

# **Variability of Microbial Taxonomic and Functional Diversities Across Management Boundaries in a Boreal Podzol**

By Abiraami Ramasamy

Submitted to the school of Graduate studies in partial fulfillment of the requirement for the degree of Masters in Science

Boreal Ecosystem and Agricultural Sciences  
Memorial University of Newfoundland

May 18, 2018  
Corner Brook, Newfoundland and Labrador

## ABSTRACT

Land capability classification describes boreal podzols as soils with severe to moderately severe limitations that restrict the capability of the land to produce crops. Nevertheless, they are used for crop production and it is predicted that more boreal podzols will be converted from forestry use to agricultural uses. This usually requires intensive conservation and fertility improvement practices aimed at correcting the excessively low pH and improving soil carbon parameters.

Under such management, it is expected that the biotic parameters and drivers of soil fertility would be drastically affected. It is hypothesized that mass and energy fluxes across the edge of a cropped field, between natural and managed conditions of soil, will alter the diversity of microbial populations and their fertility relevant functions.

To verify this, I surveyed a cropped field and its immediate surrounding areas, located within a Boreal Forest Ecosystem in Western Newfoundland. The surrounding areas, outside the four field edges covered four distinct non-cropped conditions, i.e. forested, wetland, grassland and grassed farm road border. Bacterial taxonomic diversity was assessed via a 16S rRNA obtained through an Illumina MiSeq PE 250bp amplicon sequencing of the V4 hypervariable region.

Fungal taxonomic diversity was assessed on an ITS dataset obtained through an Illumina MiSeq PE 250bp amplicon sequencing of the ITS1-2 region. A predictive functional profiling of the bacterial community, based on the 16S rRNA results (PICRUSt) was then carried out. Results are contextualized by standard abiotic soil parameters and compared to potential nitrogen mineralization rates along a management intensity gradient, i.e. a gradient crossing from natural to cropped conditions. Both surface and subsurface layers were considered. Standard and exploratory statistics were carried out and included an analysis of ecological indicators for population diversity. Statistical analysis was carried out separately on soil physicochemical properties, microbial taxonomic diversity, and microbial functional diversity. Correlational analyses between microbial diversity and physicochemical properties and were carried out separately. It was found that, while the natural conditions tested had distinct diversities, the results became increasingly similar towards the field centre, away from the natural edge. Thus, land management affects the taxonomic and functional diversity of microorganisms and also found that the shift in taxonomic and functional diversity is directly related to the distance from the natural areas.

## **ACKNOWLEDGEMENTS**

To my supervisor Dr. Adrian Unc, I would like to convey my deepest thanks for his trust, endless support and guidance throughout my research work. I would also like convey my appreciation to my co-supervisor Dr. Mumtaz Cheema for his guidance. I thank my committee members Dr. Lakshman Galagedara and Dr. Dmitry Sveshnikov for their suggestion and guidance. I thank Crystal McCall, Dr. Daniel Altdorff, Dr. Tao Yuan, Marissa O'Brien, Janelle Marchand, Candace Mangione and Nicholas Diamond for their fieldwork. My special thanks to Crystal McCall and Dr. Tao Yuan for their assistance and support in BERI (Boreal Ecosystem Research Initiative) laboratory.

## TABLE OF CONTENTS

Abstract .....	2
Acknowledgements .....	3
LIST OF FIGURES.....	7
LIST OF TABLES .....	10
1 Literature Review .....	11
1.1 Soil .....	11
1.1.1 Soil Formation .....	11
1.1.2 Soil Texture and Structure .....	11
1.1.3 Soil Air and Soil Water .....	12
1.1.4 Soil Temperature: .....	13
1.1.5 Soil Chemical Properties/ Nutrients: .....	13
1.1.6 Podzolization: .....	13
1.2 Soil Microbiology .....	14
1.2.1 Soil and Ecosystem Services .....	14
1.2.2 Resistance and Resilience .....	15
1.2.3 Soil microbial biomass, .....	15
1.2.4 Soil Enzymatic Activity .....	15
1.2.5 Bacterial and Fungal Communities .....	16
1.2.6 Ecological Niches .....	17
1.2.7 Role of Soil Depth .....	17
1.2.8 Relationship to Plant community .....	18
1.2.9 Nitrogen Cycle .....	19
1.2.9.1 Nitrogen fixation.....	19
1.2.9.2 Ammonia Oxidation.....	20
1.2.9.3 Denitrification.....	20
1.2.10 Phosphorus cycle .....	21
1.2.11 Carbon Cycle and SOM .....	21
1.2.12 Impact of Agricultural Practices .....	22
1.2.12.1 Tillage .....	22



1.2.12.2	Crop Rotation .....	23
1.2.12.3	Fertilization (Inorganic Vs Organic Fertilizers) .....	24
1.2.12.4	pH .....	25
1.2.13	Chemical Amendments .....	28
1.2.14	Soil Compaction.....	28
1.2.15	Anaerobic conditions.....	29
1.2.16	Aerobic conditions.....	31
1.3	Functional diversity .....	32
1.3.1	Amino acid production and catabolism.....	32
1.3.2	Biosynthesis of secondary metabolites .....	33
1.3.3	Lipids .....	33
1.3.4	Vitamins and co-factors.....	33
1.3.5	Carbohydrates metabolism .....	34
1.4	Soil Microbiology – Methodological approaches, molecular perspective.....	34
1.4.1	Temporal and spatial variation.....	35
1.4.2	Exchanges of mass, energy and information between managed soils and the non-managed soils surrounding agricultural plots .....	35
2	Introduction: .....	37
2.1	Newfoundland: unique scenario of land-use change in a boreal system?.....	37
2.2	Hypothesis.....	37
3	Methodology.....	39
3.1	Site Description .....	39
3.2	Site Description and Sampling Design .....	39
3.3	Sample analysis .....	42
3.3.1	Soil handling .....	42
3.3.2	Soil Physicochemical Analyses.....	42
3.3.2.1	Soil texture .....	42
3.3.2.2	pH .....	43
3.3.2.3	Available phosphorus .....	43
3.3.2.4	Survey of total cations.....	44

3.3.2.4.1	Sample Preparation and analysis on ICP-MS:.....	45
3.3.2.5	Total Carbon and Nitrogen: .....	46
3.3.3	Microbiological methods.....	46
3.3.3.1	DNA extraction .....	47
3.3.3.2	Sequencing .....	47
3.3.3.2.1	Bacterial taxonomic diversity via amplicon sequencing.....	47
3.3.3.2.2	Fungal taxonomic diversity via amplicon sequencing .....	47
3.3.3.3	Sequence Analysis: .....	48
3.3.3.4	Taxonomic diversity.....	49
3.3.3.4.1	Bacteria .....	49
3.3.3.4.2	Fungi:.....	49
3.4	Statistical Analysis .....	49
3.4.1	Physicochemical Analyses .....	49
3.4.2	Microbial Taxonomic Diversity Analysis .....	50
3.4.2.1	Alpha Diversity.....	50
3.4.2.2	Beta Diversity .....	51
3.4.2.2.1	Discriminant analysis.....	51
3.4.3	Correlation between microbial diversity and soil properties .....	52
3.4.4	Functional Diversity .....	53
4	Results.....	54
4.1	Soil Physiochemical Parameters.....	54
4.1.1	Soil Physical properties.....	54
4.1.2	Soil Chemical parameters.....	58
4.2	Similarity of the abiotic properties profiles of the sampled sites and gradient locations	75
4.3	Alpha Diversity .....	77
4.4	Beta Diversity .....	81
4.4.1	Predictive discriminant analyses .....	87
4.4.2	Relationship between biotic and soil abiotic diversities .....	93
4.5	Functional Diversity.....	101

4.5.1	Metabolic Diversity .....	101
4.5.2	Xenobiotics Biodegradation .....	105
5	Discussion .....	108
6	Conclusions .....	115
7	Reference:.....	117

## LIST OF FIGURES

Figure 1.1:	Farmland setup in Newfoundland; inadvertent Agroforestry? .....	36
Figure 3.1:	Description of sampling transects across the field edge. ....	40
Figure 3.2:	Soil sampling design .....	42
Figure 4.1:	Textural distribution of soil samples .....	55
Figure 4.2:	Sand% across the sampling gradients in both the depths at all sampling sites .....	56
Figure 4.3:	Soil texture across the sampling gradients; clay (%) in both the depths at all sampling sites .....	57
Figure 4.4:	Soil pH across the sampling gradients for both depths at all sampling sites .....	61
Figure 4.5:	Concentration of sodium (Na) in soils across the sampled gradients for both depths at all sampling sites .....	62
Figure 4.6:	Concentration of potassium (K) in soils across the sampled gradients for both depths at all sampling sites .....	63
Figure 4.7:	Concentration of calcium (Ca) in soils across the sampled gradients for both depths at all sampling sites .....	64
Figure 4.8:	Concentration of manganese (Mn) in soils across the sampled gradients for both depths at all sampling sites .....	65
Figure 4.9:	Concentration of copper (Cu) in soils across the sampled gradients for both depths at all sampling sites .....	66
Figure 4.10:	Concentration of zinc (Zn) in soils across the sampled gradients for both depths at all sampling sites .....	67
Figure 4.11:	Concentration of magnesium (Mg) in soils across the sampled gradients for both depths at all sampling sites .....	68
Figure 4.12:	Concentration of aluminum (Al) in soils across the sampled gradients for both depths at all sampling sites .....	69

Figure 4.13: Concentration of iron (Fe) in soils across the sampled gradients for both depths at all sampling sites .....	70
Figure 4.14: Total organic carbon (TOC) in soils across the sampled gradients for both depths at all sampling sites .....	71
Figure 4.15: Total phosphorus (TP) in soils across the sampled gradients for both depths at all sampling sites .....	72
Figure 4.16: Available phosphorus (PO <sub>4</sub> -P) in soils across the sampled gradients for both depths at all sampling sites .....	73
Figure 4.17: Total nitrogen (TN) in soils across the sampled gradients for both depths at all sampling sites .....	74
Figure 4.18: Similarity of soil samples' chemical-physical and textural profiles as described by Principal Component Analysis (PCA). Data for Depth 1. ....	75
Figure 4.19: Similarity of soil samples' chemical profiles across sampling sites as described by Principal Component Analysis (PCA). Data for Depth 2 .....	76
Figure 4.20: Alpha diversity indices for the bacterial community. Indices calculated using OTU abundance data.....	78
Figure 4.21: Alpha diversity indices for the fungal community, (calculated on OTU abundance). Bars describe the distance between the maximum and minimum values for each condition. ...	80
Figure 4.22: Comparison of microbial diversity along the <i>NatField</i> to <i>AgField</i> gradient; change in the Bray-Curtis similarity index value along the sampled gradient versus the -3m location. Error bars describe the 95% CI. ....	83
Figure 4.23: Comparison of microbial diversity along the <i>NatField</i> to <i>AgField</i> ; change in the Bray-Curtis similarity index value along the sampling location versus the -3m sampling location. Error bars describe the 95% CI. ....	85
Figure 4.24: Discriminant analysis of gradient locations based on the structure of the <b>abiotic factors</b> . Confusion matrices and prediction quality parameters (see Methodology). ....	89
Figure 4.25: Discriminant analysis of gradient locations based on the <b>bacterial taxonomic diversity</b> structure. Confusion matrices and prediction quality parameters (see Methodology). ....	90
Figure 4.26: Discriminant analysis of gradient locations based on the <b>fungal taxonomic diversity</b> structure. Confusion matrices and prediction quality parameters (see Methodology).....	91
Figure 4.27: Discriminant analysis of gradient locations based on the PICRUST based <b>inferred functional diversity structure</b> . Confusion matrices and prediction quality parameters. ....	92
Figure 4.28: Relationship between bacterial taxa, (class level) and abiotic parameters. East sampling site. ....	94

Figure 4.29: Relationship between bacterial taxa, (class level) and abiotic parameters. North sampling site. ....	94
Figure 4.30: Relationship between bacterial taxa, (class level) and abiotic parameters. South sampling site. ....	95
Figure 4.31: Relationship between bacterial taxa, (class level) and abiotic parameters. West sampling site. ....	95
Figure 4.32: Relationship between bacterial taxa, (class level) and abiotic parameters. Comparing <i>AgField</i> (10m) and <i>NatField</i> (-3m) at Depth 1 sample points .....	96
Figure 4.33: Relationship between bacterial taxa, (class level) and abiotic parameters. Comparing <i>AgField</i> (10m) and <i>NatField</i> (-3m) at Depth 2 sample points. ....	96
Figure 4.34: Relationship between fungal taxa, (class level) and abiotic parameters. East sampling site. ....	98
Figure 4.35: Relationship between fungal taxa, (class level) and abiotic parameters. North sampling site. ....	98
Figure 4.36: Relationship between fungal taxa, (class level) and abiotic parameters. South sampling site. ....	99
Figure 4.37: Relationship between fungal taxa, (class level) and abiotic parameters. West sampling site. ....	99
Figure 4.38: Relationship between fungal taxa, (class level) and abiotic parameters. Comparing <i>AgField</i> (10m) and <i>NatField</i> (-3m) at Depth 1 sample points. ....	100
Figure 4.39: Relationship between fungal taxa, (class level) and abiotic parameters. Comparing <i>AgField</i> (10m) and <i>NatField</i> (-3m) at Depth 2 sample points .....	100
Figure 4.40: Relationship between Bacterial Metabolic diversity across Management Intensity in all sampling sites at Depth 1 and Depth 2.....	102
Figure 4.41: Relationship between the Bacterial Metabolic diversity across Management Intensity in all sampling sites at Depth 1 and Depth 2. ....	104
Figure 4.42: Relationship between Bacterial Biodegradation of Xenobiotic across Management Intensity in all sites at Depth 1 and Depth 2. ....	106
Figure 4.43: Relationship between the Bacterial Biodegradation of Xenobiotic Biodegradation across Management Intensity in all sites at Depth 1 and Depth 2.....	107

## LIST OF TABLES

Table 1.1: Electrical Conductivity, (EC) of clay minerals (Mandal, 1998) .....	12
Table 1.2: Average composition of soil air .....	13
Table 3.1: Summary of the sampling design (n=144) .....	41
Table 3.2: Calibration/Standard Curve of standard at different concentration .....	46
Table 3.3: Minimum Precision Criteria for Blanks and K-Factors with reproducibility.....	46
Table 3.4: Summary of the taxonomic assignment success .....	50
Table 4.1: Changes in the level of similarity of bacterial communities along the sampled <i>NatField</i> to <i>AgField</i> gradients as described by the change in the Bray-Curtis similarity index value along the sampled gradient versus the -3m location .....	82
Table 4.2: Changes in the level of similarity between fungal communities along the sampled <i>NatField</i> to <i>AgField</i> ; change in the Bray-Curtis similarity index value along the sampled gradient versus the -3m location.....	86

## 1 Literature Review

### 1.1 Soil

#### 1.1.1 Soil Formation

Soil is a mineral and organic material which acts as a natural medium for the growth of plants. It is the result of pedogenetic process and environmental mechanisms driven by climate, including water and temperature, physical, chemical and the related biological activities of a range of macro and microorganisms (USDA Natural Resources Conservation Service Soils). Soil can be described in terms of physical, chemical, biological and morphological features (USDA Natural Resources Conservation Service Soils). Differences in climate, parent material, landscape position, and living organisms from one location to another as well as the amount of time the material has been in place all influence the soil-forming process (Fortner, R, 2010; Landeweert et al., 2001).

Soil is therefore a heterogeneous natural body that, under ideal conditions, comprises solids (minerals, and organic matter, 50%), liquids (25%), and gases (25%), occurs at the Earth's surface, and is characterized by either horizons, or layers, that are distinguishable from the initial material as a result of additions, losses, transfers, and transformations of energy and matter or by the ability to support rooted plants in a natural environment (Resources and Service, 1999). Soil Physical Properties

Soil physical properties such as soil particle size, proportion, arrangement and mineral composition play a crucial role in soil infiltration rate, water holding capacity, permeability, aeration, plasticity, nutrient supply and availability.

#### 1.1.2 Soil Texture and Structure

Soil texture describes the relative proportions of sand, silt and clay. Silt particles (2-50  $\mu\text{m}$  diameter) are more reactive than sand due to their larger specific surface area (i.e.  $\text{m}^2 \text{g}^{-1}$ ). Clay particles (<2  $\mu\text{m}$  diameter) are secondary minerals, the product of the weathering of aluminosilicates, and have highly reactive surfaces and the largest specific surfaces among the soil textural groups.

Soil structure is the arrangement of elementary particles (primary and secondary) into aggregates. In combination with texture, soil structure controls the soil porosity thereby affecting the soil-water relation, aeration, root penetration and the metabolic activities of soil microbes

(Chesworth, 2006).

Clays have high nutrient and water holding capacities. The former is due to their large surface charges associated with the isomorphic substitution of high valence ions, e.g.  $Al^{3+}$ , with lower valence ions, i.e.  $K^{+}$ , during chemical weathering of clay minerals and thus the more weathered clays have larger specific surface charges combined with larger specific surfaces ( $m^2 g^{-1}$ ); these charge properties are commonly described by soil's cation exchange capacity (CEC,  $cmol kg^{-1}$ ) as shown in Table 1.1.

Table 1.1: Electrical Conductivity, (EC) of clay minerals (Mandal, 1998)

Colloid Type	CEC ( $cmol Kg^{-1}$ )
Kaolinite	2-15
Montmorillonite	80-150
Chlorite	10-40
Vermiculite (Trioctahedral)	100-200
Vermiculite (Diocahedral)	10-150
Allophane	3-250
Gibbsite	4
Goethite	4

The presence of charged clay particles leads to more aggregation and soil structure formation (mainly granular), with a larger proportion of micropores; this leads to greater soil water holding capacity. Thus, clay controls most of the soil properties (Mandal, 1998). Consequently, sandy soils are highly permeable and well-drained with low water holding capacity. Thus, they require frequent irrigation compared to fine-textured soil for better growth and productivity whereas clay soil has a disadvantage of waterlogging and poor aeration and workability. The moderate fine-textured soil (loams) therefore are the best soil group for plant growth and productivity as they have an advantage of balanced proportion of sand, silt and clay (Mandal, 1998).

### 1.1.3 Soil Air and Soil Water

Soil air and water are the two components that fill the soil pores. Soil air is lighter than soil water, and it may be pushed out of the soil as more and more water fills the pores. Soil air has an impact on weathering and decomposition of minerals and organic materials and in other chemical process such as oxidation and reduction. The amount and composition of air in the soil have direct and indirect effect on plant growth. Soil air is different from the atmosphere above the soil. Soil usually contains 10-100 times more  $CO_2$ , slightly less  $O_2$  than the atmosphere, and



is saturated in water vapor (Table 1.2). The plants root and many soil microorganisms need O<sub>2</sub> for normal respiratory processes (Batten and Gibson, 1977; Russell, 1957).

Table 1.2: Average composition of soil air

	Oxygen	Carbon dioxide	Nitrogen
Soil Air*	20.65	0.25	79.20
Atmospheric air*	20.97	0.03	79.00

\* % volume (Bridges, 1978).

#### *1.1.4 Soil Temperature:*

Soil air and temperature are important parameters as they govern seed germination, plant growth and microbial activities (Chesworth, 2006). Soil temperature governs the thermal management of soil and plant growth and productivity. Apart from solar energy as a source of soil temperature, it is found that decomposition of organic matter and mineralization of nitrogen increases the soil temperature (Chesworth, 2006). Thus, in low temperature soil, it is expected the amount of organic matter will be high and also germination found to be very slow (Chesworth 2006; Mandal 1998).

#### *1.1.5 Soil Chemical Properties/ Nutrients:*

Based on their essentiality to plants, nutrients are grouped into macronutrients, i.e. nitrogen, phosphorus and potassium, and micronutrients such as iron, manganese, zinc, and copper. The total amount of nutrient content depends on the nature of the parent material and biological activities. The availability of nutrients to plants also varies with soil type and varies with depth. Chemical properties of soil largely depend on soil colloids which are the most reactive part of soil. Soil colloids are grouped into two phases as organic compounds such as fresh or decomposed debris of plants, animals and microbes, and inorganic compounds mainly composed of clay particle of variable electrostatic charges (Batten and Gibson, 1977).

#### *1.1.6 Podzolization:*

Podzol comes from Russian word “pod” meaning under or beneath and “zola” meaning ash. Most podzols are found in temperate or boreal forest regions with cool temperatures, humid or humid soil moisture regimes, and in medium to coarse textured acid parent materials generally rich in quartz. Podzols are characterized by a strongly differentiated horizon sequence with a light coloured eluvial A or E horizon which overlies a darker illuvial B horizon enriched with

aluminium and iron oxides and organic matter (Sanborn et al., 2011).

## *1.2 Soil Microbiology*

### *1.2.1 Soil and Ecosystem Services*

The terrestrial ecosystem is an important resource for almost all biotic parameters on Earth via biogeochemical cycling and climate regulation. The soil ecosystem functions and services help in food production, regulation of atmospheric greenhouse gases, regulation of water quality and maintenance of above and belowground biological communities (Finn et al., 2017).

Microorganisms play a vital role in ecosystem functions, nutrient cycling (Dobrovolskaya et al., 2015), decomposition of plant and animal residues, formation of humus (Fedotov and Lysak, 2014), and soil formation (Hobara et al., 2014), help in the conservation of organic matter, nitrogen immobilization and recycling of nutrients (Powlson, 2001), plant growth promotion (Hayat et al., 2010), control plant pathogens and weeds, and affect soil structure by the production of organic and inorganic acids, accumulation of nutrients in soil, organic pollutants mineralization and bioremediation of contaminated soils (Hayat et al., 2010)

Enormous land area has been converted to agricultural uses to meet the requirement of food and fiber. This conversion affects the soil physical properties by altering soil water capacity (Tong and Chen, 2002), soil aggregate stability, nitrogen and phosphorus, and below-ground communities (Islam and Weil, 2000), soil organic carbon and carbon storage (Houghton et al., 1999) and affects the microbial community composition and its functions (Bossio et al., 2005). Microbial diversity and community composition plays an important role in agricultural processes such as plant productivity through regulation of nutrient cycles (Finn et al., 2017). Agricultural management practices such as fertilisation helps to increase bioavailability of carbon and energy for microbial activity which results in the alteration of microbial community structure (Finn et al., 2017). The land conversion also affects the abundance of plant and animal community which in turn affects the soil ecosystem functioning (Laliberte et al., 2010).

Land use such as cropping and pasturing and land management practices such as tillage, crop rotation, fertilizer application increases the carbon bioavailability, disturbs soil aggregates, aboveground plant biomass (increase/ decrease). They also affect the quality and quantity of bioavailable soil organic matter (SOM) by decreasing the molecular complexity of plant biomass or by decreasing the SOM stability in soil via altering the pH. Change in the carbon bioavailability affects growth rate of soil microbes and also induce a narrowing of microbial

functions due to decreased Carbon Use Efficiency (CUE). CUE is the microbial efficiency when converting available organic substrates into stable biosynthesized products. Microorganisms metabolize a wide range of compounds to satisfy the heterotrophic demands for carbon and energy (Karhu et al. 2014; Frey et al. 2013). This results in higher mineralization of SOM in agricultural ecosystem compared to natural ecosystem (Finn et al., 2017). More conversion of natural to agricultural ecosystems affects the ecosystem services (Poulin and Darveau, 2016) by decreasing soil fertility, soil productivity, and bringing on a slew of environmental issues such as erosion that can also lead to eutrophication of water bodies, and increased greenhouse emissions among others (Defries et al., 2004; Matson et al., 1997)

#### *1.2.2 Resistance and Resilience*

It is very important to understand the concepts of resistance and resilience as these two aspects are directly connected to stability of soil ecosystem functions, critically so in disturbed soil ecosystem.

Resistance is the ability of a system to withstand the disturbance whereas ecological resilience is defined as amount of disturbance needed to migrate a community from one steady state to another/alternative steady state (Griffiths and Philippot, 2013).

#### *1.2.3 Soil microbial biomass,*

Soil microbial biomass (SMB) acts a sink for C and N immobilizing available mineral nutrients in unavailable organic forms. SMB comprises 2-3% and 3-5% of SOC and nitrogen, respectively (McGill et al., 1986). Lysis of microbes allows for mineralization and release of fixed nutrients. Thus, SMB acts as both source and sink of nutrients and as a catalyst for the conversion of nutrients between organic and mineral forms, the latter an available form of nutrient to plants (McGill et al., 1986). Land management practices such as tillage generally decrease the SOC in microbial biomass (McGill et al., 1986).

#### *1.2.4 Soil Enzymatic Activity*

Microorganisms produce exo-enzymes which help in soil organic matter (SOM) degradation. Microorganisms target and obtain the required substrate, carbon and nutrients through exo-enzymes. Thus, exo-enzyme activities relate to the chemical composition of SOM, carbon and nitrogen. Individual microbial abundance and diversity govern exo-enzyme production whereas the structure of microbial community is governed by environmental factors such as pH, moisture, temperature and carbon availability. Changes in environmental factors lead to shift in the

dominant microbes within a community, with an advantage for the ones that can adapt more efficiently to the new circumstances; this in turn can alter the functional processes of that community. Such shift will change the microbial enzymatic profile, and related processes and the decomposition rates of SOM (DeForest et al., 2012; Eilers et al., 2012; Kaiser et al., 2010; Schimel and Schaeffer, 2012; Sinsabaugh et al., 2009, 2008; Strickland et al., 2009; Talbot et al., 2013; Waldrop and Firestone, 2006)

### *1.2.5 Bacterial and Fungal Communities*

Soil bacterial community is the wide, abundant and diverse group of organisms in soil with an approximated  $10^{-4}$  to  $10^{-6}$  different genomes (Mader et al., 2002; Torsvik and Goksoyr, 1978). Microbial community composition is affected by many factors including soil pH, nutrient availability, toxic compounds, plant cover, soil structure and land use history and practices. These factors affect the soil bacterial and fungal community in terms of community structure and diversity (Dequiedt et al., 2011; Fierer et al., 2007; Girvan et al., 2003; Gomes et al., 2003; Halling, 2017; Lauber et al., 2009, 2008; Lupwayi et al., 1998; Nacke et al., 2011; Rousk et al., 2010). Soil environment such as soil fertility, soil type, soil pH, carbon, man-made flooding in rice field, and seasonal variation influences the bacterial activity and diversity (Bossio and Scow, 1998; Girvan et al., 2003; Schutter et al., 2001)

The soil environment is reported to harbor the majority of fungal species. Of these, 80,000 to 100,000 species were isolated and characterized while more than 1 million species are not yet described (Bridge and Spooner, 2001; Hoorman, 2011). Fungi are found to carry out about 20 different essential functions out of which the primary degradation of soil's complex organic polymers is the most important. Soil fungi interact and associate with plants, nematodes, arthropods and with other fungi (Bridge and Spooner, 2001; Hoorman, 2011). Fungi play many important roles in soil such as water dynamics, nutrient cycling and disease suppression (Bridge and Spooner, 2001; Hoorman, 2011). Fungi population is found to be dominant under no tillage compared to bacteria. They have a CUE 40-55% and can recycle more carbon and have higher C:N (10:1) and less nitrogen (10%) compared to bacteria (Bridge and Spooner, 2001).

Fungi have the ability to produce extracellular substances that help in the breakdown of complex polymers such as lignin and cellulose. Fungal hyphae also help in the transport of immobile nutrients, such as phosphorus, enhancing the reach of plants' roots (Carlile.R.W and Coules A 2012). Soil microbes helps in the breakdown of soluble and insoluble organic matter and convert

them to inorganic form, in such a way it is available to crop plants (Schimel and Bennett, 2004).

#### 1.2.6 Ecological Niches

In an ecological niche microbes respond similarly to environmental conditions and possibly compete for resources (Maire et al., 2012). The agricultural practices, such as tillage affect the available soil niches. Fungal community plays a strong role in symbiotic activities with plant roots and lignocellulose decomposition. This affects the evolutionary pressure on bacterial population. The competition between bacterial and fungal population for root exudates, cellulose and lignin results in the domination of decomposing fungal community but as well as creating a new niche for bacterial community (De Boer et al., 2005; Smith and Read, 2008). Every change in soil ecosystem affects the microbial communities to adapt to the change by altering the equilibrium among the microbial population. This results in decreased ability to properly adapt to environmental stress or adversity in the ecosystem (Mazzetto et al., 2016).

#### *Soil Microbes and Soil Health:*

Ammonium and nitrate-N are the available forms of nitrogen to plants. Microbes help in the conversion of proteins to amino acids, which in turn converted to ammonium (ammonification). Microbes such as *Nitrosomonas* and *Nitrobacter* helps in the formation of nitrate (Khan et al., 2009; Marschner and Rengel, 2007). *Pseudomonas*, *Aspergillus*, *Penicillium*, *Trichoderma* acts as P- solubiliser. Ectomycorrhizal fungi and pseudomonas acts as manganese reducers by which it can be available to crop plants (Khan et al., 2009; Marschner and Rengel, 2007)

Microorganism such as *Azospirillum* and *Cyanobacteria* fix atmospheric nitrogen in soil through symbiosis. Biological Nitrogen Fixation(BNF) involves legumes interacting with soil microbes such as *Rhizobium* and *Bradyrhizobium* (Pradesh, 2010).

#### 1.2.7 Role of Soil Depth

At every depth of soil organic matter is degraded by exo-enzymes (produced inside the cell and secreted outside the cell). Most studies show that enzyme activities mostly occur in the upper 20 cm of soil even as 60% of the carbon stored in the soil is below 30 cm (Jobbágy and Jackson, 2000; Kaiser et al., 2010; Sinsabaugh et al., 2008; Wallenstein et al., 2009). Soil depth controls mass and energy flow and thus temperature, soil moisture and pH, which are the main factors for enzymes activity and microbial growth (Rovira and Ramón Vallejo, 2002). In subsoil (below 30 cm), SOM is bound to minerals. Therefore, microbes access to this deep, chemically stable SOM is limited in subsoil. In the top soil, the proximate source of SOM is plant debris while in the

subsoil, is due to microbial activity in the top soil layer, which results in soluble SOM that eventually is leached to the mineral rich B-horizon (Wallander et al., 2003).

Through mineralization of SOM in subsoil, carbon is lost through respiration as CO<sub>2</sub> and N is recycled. This leads to lowering of C: N in soil, and microorganisms adapt to this situation by adjusting the enzyme production. The SOM enzyme activities are higher in the mineral top soil than in mineral subsoil (Rumpel and Kögel-Knabner, 2011). The functional diversity is influenced by soil depth, types of vegetation, and climate (Klimek et al., 2016). The soil surface has different physical, chemical and biological circumstances as the result of level of exposure to light, wind, erosion and agricultural management practices. Often the soil surfaces form a Biological Soil Crust( BSC) through the development of agglomerations of photosynthetic *Cyanobacteria*, algae, mosses and Lichens, which leads to an increased nutrient content compared to the bulk soil and also erosion resistance (Jeffery et al., 2009).

#### *1.2.8 Relationship to Plant community*

The change in the plant community as a result of land use change has a direct impact on composition and abundance of diazotrophs (N-Fixers). An increase in plant diversity, results in increased nitrogen mineralization and increased net nitrogen supply (Mirza et al., 2014) A combination of increased carbon content and decreased nitrogen content is suitable for nitrogen fixers. Nitrogen, C: N ratio and pH is directly affected by land use change (Mirza et al., 2014). The land use change has notable influence on below ground community (Nielsen et al., 2015) This influence is different on larger and smaller organisms in the food web (Nielsen et al., 2015).

Rhizosphere microbial community is a part of soil microbial community. Studies show that rhizosphere microbial community of different plants growing in same soil are distinct and rhizosphere community of a plant species are similar when they grow in different soil (De Deyn et al., 2008; Jassey et al., 2013; Wardle et al., 2012). However, other studies also show that different plant species that grow in same soil may have similar rhizosphere community (De Deyn et al., 2008; Jassey et al., 2013; Wardle et al., 2012). The rhizosphere microbial community structure mainly depends on the amount and composition of root exudates (De Deyn et al., 2008; Jassey et al., 2013; Wardle et al., 2012). Root exudates serves as major carbon source for microbial population, chelates nutrient in such a way that it is available to plants and helps in desorption of iron and phosphorus (less soluble) (De Deyn et al., 2008; Jassey et al., 2013;

Wardle et al., 2012). In forest ecosystems, trees influence soil by changing soil structure through the penetration of roots, increase organic carbon by root litter decomposition and root exudates. Change in vegetation, plants species and diversity has an influence on microbial community and diversity through the variation in microclimate and debris chemistry (De Deyn et al., 2008; Jassey et al., 2013; Wardle et al., 2012) and affect the soil substrate quality and quantity (De Deyn et al., 2008; Jassey et al., 2013; Wardle et al., 2012). Each forest has distinct above and belowground communities (Haichar et al., 2008; Krashevskaya et al., 2015). In boreal forests, enormous amount of organic matter is stored on the surface soil due to slow decomposition rate. Various organic matter pools are at different decomposing stages, which includes highly, moderately and minimal decomposed, septic and hemic substances. The organic surface layer and bulk mineral soil have different substrate quality by which microorganisms grow and reproduce. Therefore, the microbial communities at soil surface and in the bulk mineral soil are distinct (Chapin et al., 2002; R. He et al., 2017). Forest conversion by grazing, logging and crop cultivation leads over years to increased soil compaction, bulk density and reduced pore space (Johnson.E et al., 1989). This condition results in change of aerobic and anaerobic microbial community and eventually reduce nutrient cycles such as N, P and C (Waldrop et al., 2000).

### *1.2.9 Nitrogen Cycle*

The nitrogen cycle is microbial driven and can be simply described as three major processes, N<sub>2</sub>-fixation, nitrification and denitrification. The microorganism involved in these processes are therefore known as N-fixers, nitrifiers and denitrifiers (Stein and Klotz, 2016).

#### *1.2.9.1 Nitrogen fixation*

Nitrogen is the most important naturally available element which is essential for growth and reproduction of plants and animals (Egamberdieva and Kucharova, 2008; Shrimant Shridhar, 2012). Nitrogen is found in amino acids and proteins and also in many other organic compounds, derived from the fixation of atmospheric nitrogen (Egamberdieva and Kucharova, 2008; Shrimant Shridhar, 2012). Biological nitrogen fixation (BNF) that accounts for about 97% of total natural input to the Terrestrial Ecosystem (Vitousek et al., 1997) is mediated by microorganisms with bacteria and archaea species playing a main role.

Another major source of N input to the ecosystem is industrial fixation of N in fertilizers applied mainly to agricultural lands. Thus, while about 413 Tg of reactive nitrogen is added to the terrestrial and marine ecosystems annually, about 210 of these are the result of anthropogenic

activities (Zou et al., 2011). Anthropogenic sources account for the sum of chemical N fertilizers (the Haber-Bosch process) and the N fixed indirectly due to high energy driven reactions such as industrial activities. Nitrogen fixing microorganism (N-fixers or diazotrophs) fix molecular nitrogen ( $N_2$ ) from the atmosphere into organic N, within their biomass, and organic nitrogen (i.e.  $NH_3$ , common for symbiotic diazotrophs); the biomass fixed N pool is ammonified and available to plants upon the death and decomposition of microbial cells, while the symbiotic bacteria can provide  $NH_3$  directly to its respective symbiotic host (Zou et al., 2011). Nitrogenases are the enzymes that catalyze the process of BNF (Gaby and Buckley, 2014; Zehr et al., 2003). BNF varies greatly across ecosystems. In tropical ecosystems diazotrophs, that are capable of taking up molecular  $N_2$ , are estimated to fix 12.2 to 36.1 kg ha<sup>-1</sup>yr<sup>-1</sup> of atmospheric nitrogen (Vitousek et al., 1997). In boreal forest, most of BNF is through the association between feather moss species of *Pleurozium schreberi* and *Hylocomium splendens* and several species of cyanobacteria. Studies have shown that this association can fix nitrogen up to 4 kg ha<sup>-1</sup>yr<sup>-1</sup> in boreal forest (DeLuca et al., 2002; Gundale et al., 2015, 2011; Lagerström et al., 2007).

#### 1.2.9.2 Ammonia Oxidation

The oxidation of ammonia, obtained directly via nitrogen fixation and also secondarily via degradation of organic matter, is carried out by ammonia oxidizers. This is the first and rate limiting step in nitrification. The ammonia oxidizers convert ammonium ( $NH_4^+$ ) to hydroxylamine ( $NH_2OH$ ). This process is catalyzed by ammonia monooxygenase enzyme (AMO) which results in the subsequent formation of nitrite ( $NO_2^-$ ) (Szukics et al., 2012). Given the biological character of ammonia oxidation, or nitrification, is affected by factors affecting biological processes such as temperature, soil pH, soil water content, availability of carbon sources (Szukics et al., 2012). Depending on the abiotic conditions the oxidation may be carried out predominantly by bacteria or by archaea; for example in alkaline soil and at lower concentration of  $NH_4^+$  archaeal oxidizers, AOA, may be favoured (Marusenko et al., 2013; Wu et al., 2013).

#### 1.2.9.3 Denitrification

As a facultative respiratory pathway, denitrification is also a microbial process. Nitrate, nitrite, nitric oxide and nitrous oxide are reduced to eventually produce atmospheric dinitrogen. Incomplete denitrification, and the release of incompletely reduced gaseous nitrogen species is



the major cause for nitrogen losses from agriculture which contributes to the production of greenhouse gasses, with nitrous oxide (N<sub>2</sub>O) a most important one (Jones et al., 2008).

#### *1.2.10 Phosphorus cycle*

Phosphorus (P)-cycle in soil is an integrated process involving soil, plants and microbes. The mineral phase of P mainly consists of calcium, iron and aluminium phosphate, depending on the pH of the soil (Usda, 1994). The most labile pool of P in soil consists of phosphate ions in the soil solution, with the greatest availability at pH ranging from 6.5 to 7.5; below 5.5 and between 7.5 to 8.5, the P-availability is limited due to fixation by aluminium and iron, and calcium respectively (Usda, 1994). In soil, 20-80% of total P is in the form of organic P (Marschner and Rengel, 2007). Organic P includes labile phospholipids, inositols and fulvic acids and humic acids (Sharpley, 1995). Phosphorus is made available for biological uptake in the form of phosphate ion (PO<sub>4</sub><sup>-</sup>). Phosphate ions are very reactive and thus immobile in most soils making phosphorus unavailable to roots through simple diffusion along a concentration gradient, as is common for nitrates (Sharpley, 1995). Phosphorus transport is thus facilitated by mycorrhizal fungi which are able to explore a volume of soil larger than the one explored by plant roots (Sharpley, 1995).

#### *1.2.11 Carbon Cycle and SOM*

In the boreal ecosystems, change in the land use from forest to agriculture increases the mineralization of carbon and nitrogen (Grünzweig et al., 2003). Moreover any supplementary source of nitrogen such as fertilizer and excess atmospheric nitrogen deposition, has a direct, positive impact on carbon loss from the ecosystem (Grünzweig et al., 2003). Soil aeration through tillage also favors loss of soil carbon associated with enhanced activity of aerobic microorganisms. Drainage also leads to enhanced aeration and thus enhanced oxidation and thus loss of SOC (Bagchi et al., 2017; Gougoulas et al., 2014). Microbes control the chemical processes leading to the breakdown of SOM and also act as the largest pool of sequestered C in the soil (Schimel and Schaeffer, 2012), pH and salinity also affect total soil carbon, in a negative correlation (Rath and Rousk, 2015; Trivedi et al., 2016).

In natural soil-plant systems, 80-90% of soil nutrients are obtained through microbial degradation of plant litter (Bardgett, 2005). SOM creates conditions favorable to many soil functions relevant to nutrient retention, soil aggregation and associated enhanced water holding capacity. Intensive agriculture leads to a decline of SOM; examples are overgrazing, tillage and

land use conversion. This decline leads to reduced soil fertility, increased erosion and increased use of chemical fertilizers (Mccauley et al., 2017; USDA, 2016).

SOM content may be described as the balance between the amount of organic residues present in soil and the decomposition rate. The decomposition rates, as hinted above, depends on soil texture, drainage, C: N of organic residues, climate, cropping pattern and the forms of SOM (DOM, Dissolved Organic Matter, or POM, Particulate Organic Matter). Soils with high clay content tend to have more SOM compared to sandy soils, as clays-humic complexes act as a physical protector of SOM. The small sizes of soil pores in clay soils also limits oxygen diffusion and thus limits organic matter oxidation (Mccauley et al., 2017; USDA, 2016).

#### *1.2.12 Impact of Agricultural Practices*

Farming is estimated to be approximately 12,000 years old, as shown by evidence to cultivation of crop plants by humans (Pradesh, 2010). This process occurred at first after clearing or burning of natural vegetation such as grasslands and forests (Pradesh, 2010). Intensive agricultural practices, which include chemical fertilization, tillage and drainage among others, alter biogeochemical processes and lead to soil degradation through erosion, loss of SOM, decreased soil nutrients, increased greenhouse gas emission, compaction that eventually lowers land productivity (Balmford et al., 2010). In extreme situations, it may also lead to desertification and decrease in biodiversity. About 38% of total agricultural lands, 21% of pasture lands and 38% of forests lands around the world are considered as degraded (O'Donnell et al., 1994; Thomasson, 1992; Utuk, 2015). Agriculture has an impact on ecosystem's biodiversity which affects both the above and below ground ecosystems, affecting soil microorganisms, which in turn leads, in a feedback loop, to decreased SOC and loss of microbial diversity (García-Orenes et al., 2013; Lauber et al., 2013; Ramirez et al., 2012).

Reversal of intensive agriculture to practices that include reduced tillage, such as zero tillage or minimum tillage and accelerated replacement of SOM have been shown to increase microbial activity (García-Orenes et al., 2013; Zornoza et al., 2009). Abundance of microbes, especially bacteria, can be greater under such conditions (García-Orenes et al., 2013; Zornoza et al., 2009).

##### *1.2.12.1 Tillage*

Tillage makes soil susceptible to erosion and loss of SOM (Hobbs et al., 2008). Researchers have found that no till (NT) practices help to improve soil health and the microbial biomass and relative abundance in surface soil mainly because of litter residues (Chen et al., 2014; Govaerts

et al., 2007; Mathew et al., 2012; Nivelles et al., 2016; Plaza-Bonilla et al., 2010; Shi et al., 2012). This improves the soil water content, SOC, the C: N ratio and soil physical characteristics such as bulk density, porosity and soil penetration resistance (Chen et al., 2014; Govaerts et al., 2007; Mathew et al., 2012; Nivelles et al., 2016; Plaza-Bonilla et al., 2010; Shi et al., 2012). NT was reported to improve soil stability and to protect macro aggregates when wetting process continues over a long period of time whereas the micro aggregates are less influenced by tillage. The increase in SOC is not only due to the increase in soil aggregate stability but also to enhanced soil moisture storage capacity (Douelle, 2014).

#### *1.2.12.2 Crop Rotation*

Crop rotation (e.g. CSW- Corn, Soybean and Wheat compared to CC- Continuous Corn and CS- Corn and Soybean) significantly increases the soil microbial biomass ( $C_{mic}$ ) and the soil biological quality index (SBQI). SBQI refers to many soil components and processes related to organic matter cycling such as total organic carbon and nitrogen, microbial biomass, mineralizable carbon and nitrogen and enzyme activities (Gregorich et al., 1997). SBQI such as  $C_{mic}$  and SBQI decrease with increase in depth with the exception of the respiration quotient (microbial respiration per unit microbial biomass,  $qCO_2$ ). Total and active carbon, total nitrogen and soil chemical quality (SCQ) are enhanced. SCQ refers to the chemical speciation of nutrients and contaminants in soil which are controlled by chemical reactions occurring in soil environment and it depends on soil chemical attributes such as mineralogy, SOM, pH, Electrical Conductivity (EC) and cation exchange capacity (CEC) (Gregorich et al., 1997). The soil chemical quality index (SCQI), total and available carbon and total nitrogen was found to decrease with increase in depth under CR. Rate of soil biological quality change was also found to be higher under CR (Topp et al., 1997). CR has no significant change in the total soil porosity whereas soil aggregate stability and particulate organic matter increased by 15% and 8%, respectively (Topp et al., 1997). Soil physical quality index (SPQI) refers to mass and energy transfer and storage properties that permit water, dissolved nutrient, and air contents appropriate for maximizing crop development while minimizing environmental degradation, as well as adequate soil strength for maintaining structure and allowing root growth (Topp et al., 1997). SPQI parameters such as total porosity and soil aggregate stability decrease with increase in soil depth. The composite soil quality index (CSQI), a composite of SBQI, SCQI and SPQI, increased under crop rotation and was found to decrease with increase in soil depth. Under crop

rotation of CSW, the increase in the soil micro aggregates is mainly by root exudates and by enhanced microbial activities crop Rotation of CSW. The same trend was also found under no tillage when compared with conventional tillage practices (Aziz et al., 2013). Several studies have shown that soil aggregate stability is mostly influenced by amount of SOM addition in soil than by SOM quality (Abiven et al., 2007; Aziz et al., 2011; TISDALL and OADES, 1982). The significant change in soil quality indicators under crop rotation over a long period is characteristic to the type of crop residue, soil microclimate and microbial food web status. The low  $qCO_2$  under crop rotation reveals that microbial communities need labile carbon for metabolism, growth and activity. The low  $qCO_2$ , i.e. lower respiration per unit microbial mass and higher  $C_{mic}$  also proves that under crop rotation, where there is no tillage, implies more efficient and stable ecosystem (Aziz et al., 2011; Wardle and Ghani, 1995). Under conventional tillage, the combination of high  $qCO_2$  and low  $C_{mic}$  as an indicator of soil ecosystem stress like due to physical disturbances (Aziz et al. 2011).

In southern Ontario, Canada, NT in combination with crop rotation (Soybean- Winter Wheat > Soybean - Corn > Soybean- Winter Wheat- Corn> continuous corn) was reported to increase the total N and SOC, C and N storage (Van Eerd et al., 2014). NT alone in a Corn - Soybean rotation improved the N status of plant compared to conventional tillage in maize (Kovács et al., 2014); microbial abundance increased with greater plant diversity in rotation. Findings show that under NT and crop rotation, the abundance of microbes has increased in the surface soil and their function has improved in up to 20 cm in the soil (Sun et al., 2016). However, some finding shows that NT led to no significant effect/ difference in the microbial community abundance and function or soil structure compared to tilled soils (Bartlová et al., 2015; Plaza-Bonilla et al., 2010; Sapkota, 2012; Spedding et al., 2004).

#### *1.2.12.3 Fertilization (Inorganic Vs Organic Fertilizers)*

Many findings reported that mineral fertilizers, especially nitrogen-based, tend to affect the microbial community whereas organic nitrogen fertilizers tend to improve the biological activity and soil carbon and nitrogen content. Organic manure application helps to increase the microbial enzymatic activity in the soil and increases availability of nitrogen and phosphorus (Gryndler et al., 2006; Lazcano et al., 2012; Yu et al., 2012).

Long-term fertilization has an impact on soil chemical properties. Mineral fertilizer found to

increase the soil pH compared to organic fertilizers. Organic fertilizers found to increase the stock of SOC compared to mineral fertilizers. Fertilization was found to increase the total primary nutrients such as nitrogen and phosphorus. Soil microbial biomass carbon and dehydrogenase activity has increased as result of long-term organic fertilizer (pig manure) application compared to mineral fertilizer. Studies revealed that long term fertilization results in the increased soil bacterial and fungal diversity and taxonomic richness. Mineral fertilizers have increased the abundance and diversity of *Acidobacteria*, *Gammaproteobacteria*, *Mortierella*, *Knufia petricola* and *Zygomycetes* whereas organic fertilizers have increased the *Firmicutes*, *Cyanobacterium*, *Myxococcales*, *Acidobacteria*, *Betaproteobacteria*, *Mortierella*, *Aleuriaaurantia* and *Chytridiomycota*, also suppressed the *Nectria sp.* Community (Luo et al., 2015; Stackerbrant and Goebel, 1994). Studies have shown that long term mineral fertilizers found to alter the microbial community composition and diversity. mineral fertilizer has a negative impact on soil bacterial composition and diversity. pH and SOM have negative impact on *Acidobacteria* and are positively correlated with bacterial diversity (Yu et al., 2016). Studies have found that nitrate and ammonium fertilizer stimulated the consumption of methane in forest and rice soils (Mohanty et al., 2006). Available P, NO<sub>3</sub>-N were found to have positive correlation with organic fertilizer application where the OM is high. Fresh organic fertilizer found to increase the microbial biomass. The high abundance of Fungi in organic fertilizer due to their decomposition and OM forming ability which results in the high OM content in the soil. Microorganisms also found to have more stress under mineral fertilizer application compared to organic fertilizer application (Y. H. Lee et al., 2013).

Mineral fertilizer (urea) alter the microbial abundance. Urea application results in the increase of ammonium and nitrate over a period of time. This process leads to increase the nitrate reducing bacteria results in the production of harmful compounds such as nitrogen dioxide, nitrogen oxide and nitrous oxide which in turn indirectly suppresses the methanogens (Fan et al., 2016). Also, urea application results in decreased ammonia oxidizing archaea (AOA) and increased ammonia oxidizing bacteria (AOB) in rice field. However, the functional composition of bacteria did not change under rice field (Fan et al., 2016).

#### 1.2.12.4 pH

The soil pH reflects soils forming factors such as parent material and weathering processes associated with release of cations. Over a period of time, free cations, oxides and carbonates

leach or are removed by plant uptake (USDA Natural Resources Conservation Service 2011). Continuous formation of carbonic acids acidifies soils. For acid soils the crop growth, nutrients and yield increase with increase in pH towards a neutral range (6-7.5). In acidic soil, nutrients such as calcium, magnesium and phosphorus are found to be in deficit whereas aluminium, iron and manganese found to abound sometimes at toxic levels (USDA Natural Resources Conservation Service 2011). Bacterial growth increases with increase in pH whereas fungi can adapt to a wide range of pH (acidic-alkaline). Nevertheless, most microorganisms survive and function best at an optimum pH; this is around 7 for bacteria, 5 for fungi and >7 for cyanobacteria (USDA Natural Resources Conservation Service 2011). At very high or low pH, the microbial activity is impeded slowing down organic matter mineralization (USDA Natural Resources Conservation Service 2011).

Land conversion also results in severe change in soil pH over years. This change is caused by several processes such as cations removal by plant uptake, leaching, high water percolation and infiltration rate, and loss of OM, or by cation addition in inorganic, organic fertilizers, and chemical amendments, positive changes in OM content (USDA Natural Resources Conservation Service 2011).

Studies show that at low pH (5.2), bacterial diversity and evenness were high compared to a neutral soil (7.7) (Cho et al., 2016) whereas fungal growth increases with decrease in pH from 8.3 to 4.5 (Rousk et al., 2009). At high pH, the dissimilarity of bacterial composition is smaller. For example, *Bacterioidetes*, *Actinobacteria* and *Proteobacteria* relative abundance increase with increase in pH (4.5 to 8.3). *Mitosporic basidiomycetes* abundance increases with decrease in pH (8.3 to 4.5). The bacterial diversity is higher in pH ranges of 4-7 (Brookes et al., 2010). Bacterial growth is faster at the pH of 7, below which the growth started to slow by 80% under Leu (leucine) and dT (Thymidine) incorporation, bacterial growth was high whereas fungal growth under acetate incorporation into ergosterol, the fungal growth increases with decrease in pH. The bacterial activity is higher at higher pH (Fernández-Calviño and Bååth, 2010; Rousk et al., 2009). Soil pH has a significant effect on soil microbial composition, enzyme activities, respiration, metabolic quotient in crop lands and anthropogenic forests (Aciego Pietri and Brookes, 2009; Cao et al., 2016; Reth et al., 2005; Wittmann et al., 2004). In acidic soil, the nitrification rate is higher. AOA contribution to ammonia oxidation is higher than AOB under acidic soils (Li et al., 2018). In natural forest, heterotrophic nitrifiers contribute to nitrification

(Li et al., 2018). Studies found that under low pH, microbial population is higher in arable soils of tea gardens and vegetable farm and low under anaerobic condition of rice field and oil drilling area. Nitrate reductase activity was higher under anaerobic condition of rice field whereas alkaline phosphatase activity is higher in botanical garden and in rice field which has higher litter composition of plant residues and straw (Nath and Samanta, 2012). Microbial activity, Nitrate reductase activity and alkaline phosphatase activity was low in oil drilling area due to more acidic nature because of the formation of toxic acids and by oil spills (Nath and Samanta, 2012). At low pH, nitrification and nitrogen fixation is inhibited. pH plays an important role in herbicides and insecticides mobility and heavy metals solubility, availability of cations, aggregate stability, also survival of some plant disease causing organisms. For example: *Gaeumannomyces graminis* which infest wheat, barley, rye and several grasses is favored by alkaline pH (USDA Natural Resources Conservation Service 2011).

Soil pH affects the fungi bacterial ratio. Fungi are more tolerant to acidic conditions compared to bacteria, leads to fungal community dominance in acidic soil. Apart from pH, moisture plays a crucial role in microbial community. Bacteria is more sensitive to change in moisture content compared to fungi because of the presence of chitinous cell wall in fungi makes it tough to change in moisture content and temperature (Fierer et al., 2009; Fierer and Jackson, 2006; Holland.A.E and Coleman.C.D, 2015; Joergensen and Wichern, 2008; Rousk et al., 2009; Stevenson et al., 2014).

### *Liming*

Liming has a positive correlation with pH, microbial biomass, and phosphatases and dehydrogenases activities overtime (>1 year) (Bezdicsek et al., 2003). However while liming is associated with microbial activity the change in microbial mass is unpredictable (Bezdicsek et al., 2003). Liming is the most common practice to increase soil pH in acidic soils (Mccauley et al., 2017). Liming supplies two major nutrients to crops, calcium and magnesium. By regulating soil pH, plant diseases, such as potato scab, can also be controlled (Lawton and Kurtz, 1957).

Liming can influence solubility of many compounds. For example, under acidic condition, large amount of iron, aluminium and manganese may be into soluble and thus available form. High levels of most of these elements are toxic to plants.

Liming affects phosphorus forms in soil. Phosphate ions are highly reactive under acidic pH and form aluminium and iron phosphates which are complex stable and plant unavailable forms (i.e.

chemically immobilized). Liming of acid soils brings pH closer to neutral where P reaches its maximum solubility and thus availability to plants (Lawton and Kurtz, 1957).

#### 1.2.13 Chemical Amendments

Microbial enzymatic system covers 60-90% of the total metabolic activity in soil. Microbial composition, diversity, enzymatic activity and their functions act as a bio indicator of changes happening in soil due to agricultural practices such as application of herbicides and pesticides. Chemical amendments such as pesticides, insecticides and herbicides have a negative impact on microbial diversity within a short period of time (7-30 days), affecting the function and composition of microbial community (Muturi et al., 2017).

Microorganisms play an efficient role in the degradation of chemical substance such as aliphatic, hydroxyl and aromatic compounds through metabolic and catabolic pathways (Cook and Hütter, 1981; Milosevic and Govedarica, 2002; Seo et al., 2009). Nevertheless, excessive application of herbicides affects the microbial physiology (Cook and Hütter, 1981) and enzymatic activity by affecting biosynthesis mechanisms including protein biosynthesis, cellular membranes, production of plant growth regulators such as gibberellins (GA) and indolacetic acid (IAA) synthesis (Milosevic and Govedarica, 2002). Soil microbes use chemical amendments and their metabolites as the source of biogenic elements such as carbon and nitrogen. Some of the herbicides decomposing microbes are *Arthrobacter*, *Pseudomonas*, *Bacillus*, *Actinomycetes*, *Mycoplana*, *Agrobacterium*, *Achromobacter*, *Rhizobium*, *Corynebacterium*, *Arthrobacter*, *Flavobacterium*, *Nocardia*, *Trichoderma*, *Rhizopus japonus*, *Aspergillus* ssp., *Penicillium* ssp. and *Metharizium anosoplie* (Miskovic et al., 1983). Decomposition of pesticide mainly depends on the abundance and composition of microbial population. Impacts on the morphology and composition of microbes by chemical amendments such as herbicides depends on the composition and dosage of the chemicals and also microbial group present in the soil (Miskovic et al., 1983). Adsorption and desorption of chemical molecules depend on the physical and chemical properties of soils such as pH, OM content, CEC, texture, moisture and temperature. For example, moisture content and temperature have positive impact on the degradation of atrazine and 2,4-D. High doses however may also cause complete demise of susceptible group of microbes (Willems et al., 1996).

#### 1.2.14 Soil Compaction



In healthy soil ecosystems, particles bind together into a stable aggregate via fungal hyphae, microbial, plant root, and earthworm exudates which in turn improve soil structure which improves infiltration rate, protects soil from erosion, crusting and compaction (USDA, 2004). Earthworm and other burrowing creatures helps in the movement of water through the soil through the formation of macropores. As mentioned above, in soil ecosystems, microorganisms help reducing the impacts of pollutants by buffering, detoxifying and decomposing the pollutants (USDA, 2004). Some microbes therefore help in the reclamation of contaminated soil and water bodies. Soil have approximately 50-60% of the volume as pore space (USDA, 2004). Compaction reduces the range, continuity and tortuosity characteristics of the pore size distribution and alters the movement of air and water in soil, which in turn leads to a shift in the microbial community from aerobic towards anaerobic (Jordan et al., 2003). The later conditions favor denitrification. Compaction can therefore cause drastic change in soil moisture and temperature fluxes and profile which governs the microbial abundance and activity in soil and nutrient release to plants. Compaction reduces the capacity of soil to store and transport water or to hold air which results in water runoff, leaching to nutrients, and increased erosion in steep slopes. Compaction also limits the root penetration depth, which supports microorganisms (Jordan et al., 2003). Sandy loams, loams and sandy clay loams are more susceptible to compaction (USDA, 2001).

#### *1.2.15 Anaerobic conditions*

Nitrification and denitrification govern bioavailability of nitrogen (Van Der Heijden et al., 2008). Approximately 98% of soil nitrogen comes from dead organic matter in the form of complex insoluble polymers such as proteins and nucleic acids which are broken-down to DON (Dissolved Organic Nitrogen) by microbes using exoenzymes (Schimel and Bennett, 2004). Soil anaerobic condition have negative impact on plant productivity, organic matter content and nutrient dynamics. Anaerobic conditions mostly occur in flooded and poorly drained, compacted soils. In well drained soils, anaerobic/ anoxia is limited in time and space. Anaerobic, reducing conditions, is associated with reduction of  $\text{Fe}^{3+}$  and  $\text{Mn}^{4+}$ , denitrification, fermentation, nitrate respiration, sulfate reduction,  $\text{CO}_2$  reduction, acetate splitting and proton reduction (J. M. Tiedje et al., 1984). Some anaerobes play an important role in pollutant degradation, including pesticides and xenobiotics. Denitrification and fermentation is mostly carried out by facultative

anaerobes in soils (J. M. Tiedje et al., 1984). Aerobic and anaerobic conditions vary on a gradient with depth and are greatly influenced by tillage. For example, in no tillage aerobes and anaerobes population are found to be greater in top 7.5 cm compared to conventional tillage whereas for the 7.5 cm to 30 cm layer the trend was totally reversed (aerobes and anaerobes population higher in conventional tillage) and facultative anaerobes did not follow any trend (Linn and Doran, 1984). Denitrification under anaerobic conditions is also influenced by pH (positive correlation), drainage (fallow land > poorly drained soil > wetland) and fertilizer type (combination of fertilizer with animal manure > calcium ammonium nitrate)(Hofstra and Bouwman, 2005).

Potential methane production is high under anaerobic conditions, in chemotrophic natural wetlands, oligotrophic natural wetlands, rice paddy field and in landfills. Methanogens are sensitive to temperature and oxygen. Methanogenic bacteria are highly active under the optimum pH of 7.0 and in the temperature range of 20-40 °C (Topp and Pattey, 1997). Roots and rhizomes can affect methane production via root decaying process and root exudates which has been found in wetland plants such as *Calamagrostis canadensis* and *Typha latifolia*. In paddy field, root associated methane production contributes up to 52% (Segers, 1998). *Methanobacterium*, *Methanosarcina*, *Methanosaeta* and *Methanospirillum* are known methanogenic bacteria (Le Mer and Roger, 2001). Mechanism of slope position also affects the methanogenic and methanotrophic activities as for higher slopes soil, water and OM will be lost and accumulates in lower slopes. This leads to wet moisture regime for prolonged period where oxygen concentration is significantly less in lower slope or depression leads to increased methanogenic and methanotrophic activities (Brzezińska et al., 2012).

Sulfur cycling mainly depends on soil texture and moisture as these parameters plays an important role in soil aeration. Sulfur oxidation takes place under aerobic condition whereas sulfur reduction takes place under anaerobic condition. The inorganic forms of sulfur are sulphate in aerobic condition and sulphide in anaerobic condition. Sulphates are mostly water-soluble salts such as sodium, magnesium and calcium sulphates while some are insoluble sulphates such as sulphate as impurity in calcium carbonate in calcareous soils. The soluble sulphates generally increase with increase in depth as the results of leaching and drainage. Podzolic soil contains total sulfur of about 0.1-1.5 tones ha<sup>-1</sup> (Konstanz, 1981). Under arable condition, formation of sulphates with lower oxidation states are very low whereas under

anaerobiosis they increase (anaerobiosis is the process in which oxygen consumption rate exceeds the supply rate due to high moisture content as the result of waterlogging condition). Microaerobic or anaerobic conditions may lead to several negative impacts to soil such as N loss by denitrification, production of organic acids and hydrogen sulphide. This condition also favors N<sub>2</sub>-fixation by free-living heterotrophs, slow down the mineralization process of OM, increase the degradation of pesticides such as DDT and lindane. Under anaerobiosis, denitrification and fermentation are the most important processes driven by facultative anaerobes (J. M. Tiedje et al., 1984), especially in tidal swamps, a considerable amount of sulphides is formed which are mostly confined to subsoils below the water table. Under tidal marsh, accumulation of sulphur is mostly sulphides and polysulphides of iron due to reduction process (Konstan, 1981). Apart from sulfur reduction, iron and Mn translocation is predominantly occurs under anaerobiosis (Stolzy and Fiehrer, 1978).

Sulfur oxidizing bacteria (SOB), sulfur reducing bacteria (SRB), and iron-reducing bacteria (IRB) are found in acidic landfills cover soil (pH 4.7- 5.37) and in coastal acid sulfate soils. Some of the SOB are *Halothiobacillus*, *Thiobacillus*, *Thiovirga* and *Bradyrhizobium* whereas SRB are *Desulfobacca*, *Desulforhabdus* and *Syntrophobacter* and IRB are *Geothrix*, *Bacillus*, *Geobacter*, *Desulfuromonas* and some SOBs (Ling et al., 2015; Xia et al., 2014). Sulfur reducing microorganisms like *Desulfosporosinus* spp are found in wetlands of rice cultivation (Pester et al., 2012).

#### 1.2.16 Aerobic conditions

Soil pH, total carbon and moisture play an important role (positive correlation) in ammonification process (Wolińska et al., 2016).

Under aerobiosis, nitrate is formed which is then denitrified in the intermediate period of anaerobiosis (Ulehlova, 1988).

Nitrification can occur only under aerobic condition where the dissolved oxygen (DO) level is 1 ppm or more. If DO is less than 0.5 ppm, it restricts the growth of nitrifying microbes (Johnson, 2011).

Nitrification is the process by which ammonium (NH<sub>4</sub><sup>+</sup>) or ammonia (NH<sub>3</sub>) is converted into nitrite (NO<sub>2</sub><sup>-</sup>) called ammonia oxidation which is a rate limiting process and nitrite to nitrate (NO<sub>3</sub><sup>-</sup>) is called nitrite oxidation. This process is rapid in warm, moist and aerated soil and slow

<10°C soil temperature (Wiederholt and Johnson, 2005). Nitrifying organisms are usually heterotrophic and use carbon dioxide as their energy source (Wiederholt and Johnson, 2005). In nitrification process, ammonia oxidation is performed by AOB such as *Nitrosomonas* spp. and *Nitrospira* spp. of Betaproteobacteria and *Nitrococcus* spp. of Gammaproteobacteria and by AOA (Xia et al., 2014).

Nitrite oxidation is performed by Nitrite Oxidizing Bacteria (NOB). Some of NOB are *Nitrobacter* spp. of Alphaproteobacteria, *Nitrospira* spp. of Deltaproteobacteria, *Nitrococcus* spp. of Gammaproteobacteria and *Nitrospira* spp. of Nitrospira. *Nitrospira* spp. dominates Nitrite oxidation in paddy fields, wastewater and in agricultural grasslands (Ishii et al., 2011) under microaerophilic condition where the oxygen level is low.

### 1.3 Functional diversity

#### 1.3.1 Amino acid production and catabolism

Under low temperature, low availability of inorganic N is due to slow decomposition. In such conditions, amino acids and peptides act as a source of N for microorganisms for growth and energy (Atkin, 1996). L-Amino acid is the key component of nitrogen cycle in soil in association with non-mycorrhizal plants such as wheat and arctic sedges and also found to be transported through plant roots (Chapin III et al., 1993; Hill et al., 2011b; Nasholm et al., 2008; Tegeder and Rentsch, 2010). The D & L notations on amino acids and other compounds like carbohydrates refer to a property known as stereoisomerism, where compounds are identical in atomic structure, but are non-super imposable mirror images of each other. The L and D in front of an amino acid is a shortened scientific notation for “levorotatory”, ‘dextrorotatory’ respectively. D-amino acids are found in some of bacterial cell wall as D-alanine and D-glutamic acid, or D-aspartic acid (Vranova et al., 2012). Bacteria can produce 10 different kinds of D-amino acids such as D-alanine and D-glutamate. Both D and L-amino acids occur as free amino acids and/or bound to soil OM (Vranova et al., 2012). L-Peptides can be taken up by plant and microorganisms (Hill et al., 2011a; Tegeder and Rentsch, 2010). D-enantiomers of amino acids are also a source of N used by plants and microbes (Dowd and Hopkins, 2009; Hopkins et al., 1997). D-amino acids are utilized by microbes as N-sources but not by plants (Hill et al., 2011b). In Maritimes Antarctic, it was found that microbial communities have the capability to metabolize D-peptides (Hill et al., 2011b; Wilkinson et al., 2014). Studies also shown that alanine is mostly used up by gram positive bacteria whereas L-enantiomers are utilized by a wide

group of both bacteria and fungi (Broughton et al., 2015).

### 1.3.2 Biosynthesis of secondary metabolites

Secondary metabolites are not intrinsically necessary for growth and development. However, many secondary metabolites help microorganism adapt and compete. For example some help form symbiotic relationships with plants and higher animals (Karlovsky, 2008). Fungi and *actinomyces* are prolific producers of secondary metabolites; penicillin, erythromycin and streptomycin which act as antibacterials are produced by *Penicillium chrysogenum*, *Saccharopolyspora erythraea* and *Streptomyces griseus* respectively. Clavulanic acid produced by *Streptomyces clavuligerus* act as enzyme inhibitors. Cephalosporin produced by *Streptomyces* spp acts as antibiotic under anaerobic condition (Barrios-González et al., 2003).

### 1.3.3 Lipids

Plants are major producer of lipids. Animals also produce lipids. Plants require lipids for signaling, membrane biogenesis and to store carbon and energy. In some plants, it helps to protect them from desiccation and infection (Schmid and Ohlrogge, 1973). Fungi store energy in the forms of neutral lipids such as triacylglycerol (Joann and Luis, 2009). The lipids are classified as storage/simple (neutral) lipids as triacylglycerol, membrane/compound (polar) lipids such as phospholipids and glycolipids and derived lipids such as cholesterol and carotenoids (Fahy et al., 2014).

In soil, 20% of humus is in the form of lipids. This value is higher in podzol and peat soils. Lipids are of different types based on their composition and function such as paraffin, hydrocarbons, carotenoid, phospholipids, fats, waxes, fatty acids and terpenoids. They play different roles such as phytotoxic effects, example Gibberellins (diterpenes) produced by plant fungus and some may cause depression in seed germination (Stevenson, 1966). Plant derived lipids present in the soil due to incorporation by above ground biomass whereas root-derived lipids contribute to SOM (Wiesenberg et al., 2010). Phospholipids fatty acids are the key component of microbial cell membrane and it is widely used to identify the microbial community structure in soil (Quideau et al., 2016). Sterols are found in plants, bacteria (methylophs) and in fungi (*Mucor* spp.) (Harwood, 1984; Jones, 1970).

### 1.3.4 Vitamins and co-factors

Soil contains vitamins and co factors which are used up by microbes for their growth. In rhizosphere region, microbes found to produce large amount of growth factors. The amount of

free vitamins present in the soil depends on the assimilation and decomposition rate (Quastel, 1965).

#### *1.3.5 Carbohydrates metabolism*

Carbohydrates is classified into three major classes based on the number of sugars as monosaccharides (one sugar molecules), oligosaccharides (two to ten monosaccharides) and polysaccharides (> hundred monosaccharides) (Clara et al., n.d.). Carbohydrates constitute about 5-25% of organic matter in soils (Cheshire, 1979). Land use change leads to significant decrease in carbohydrates due to increased mineralization and tillage and decreased organic matter (Plante et al., 2005). Studies found that in cultivated area of tropical climate, carbohydrates are higher due to increased soil litter compared to forest (Ratnayake et al., 2013). Carbohydrates metabolism by microbes play an important role in carbon and nitrogen cycles in soils (Gianfreda and Bollag, 1996). Plants and plant parts are the main sources of carbohydrates in soil. Carbohydrates also play a role in N cycle (Quastel, 1965). Arabinose and xylose which are derived from plants serve as energy source for microorganism whereas galactose, mannose, rhamnose and fucose are released from microbes to soil (Cheshire, 1979; Murayama, 1984).

#### *1.4 Soil Microbiology – Methodological approaches, molecular perspective*

Total number of operational taxonomic units (OTUs), in a single soil habitat is huge ranging up to millions (Gans et al., 2005; Schloss and Handelsman, 2006) Soil is considered to have a large proportion of genetic diversity on earth, thus the microbes establish a wide range of metabolic process in soil ecosystem (Whitman et al., 1998).

There are different databases for microbial diversity such as Ribosomal Database Project (RDB)- 16SrRNA database (“RDP,” 1992), SILVA - rRNA database (<https://www.arb-silva.de/>), Greengenes - 16SrRNA database (“Green Genes,” 2005), BIGSdp, Bacterial Isolate Genome Sequence database (Jolley, 2010), European Bioinformatics Institute (EBI)- metagenomics portal for submission and analysis of metagenomics data (“EMBL- European Molecular Biology Laboratory European Bioinformatics Institute (EMBL-EBI),” 1980), UNITE, Unified system for the DNA based fungal species -ITS, Internal transcribed spacer (“Unite community,” 2012). Microbial community functions cannot be studied directly by profiling phylogenetic marker genes such as the 16s rRNA. However, PICRUST (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States), a computational approach, used in this study, predicts the

metagenomic functional composition using 16S rRNA and Greengene database. PICRUST currently can only use an OTU table with Greengenes OTU identifiers which is the output from closed-reference picking or by filtering out de-novo OTUs after open-reference picking (Langille et al., 2013).

#### *1.4.1 Temporal and spatial variation*

Microbial community is subjected to both temporal and spatial variations. Temporal variability is the temporary change in the structure of microbial community of a given site due to succession and evolutionary change (Bargett et al., 2005). Spatial variability is the change in the structure of the microbial community of an agricultural landscape across a distance due to management practices such as tillage and fertilizer application (Naveed et al., 2016; Peigné et al., 2009). For example, in a temperate forest ecosystem in Norway, tree distance and seasonal variation plays an import role in bacterial and fungal community composition. Bacterial community was found to respond more quickly to stressors than fungal community (Nacke et al., 2016). Exploration of the  $\alpha$  and  $\beta$ -diversity profiles can help understanding the temporal and spatial variation of the soil microbial community. The  $\alpha$ -diversity represents the total microbial diversity within a given site whereas  $\beta$ -diversity represents the total microbial variation between different sites (Bargett et al., 2005).

#### *1.4.2 Exchanges of mass, energy and information between managed soils and the non-managed soils surrounding agricultural plots*

Agroforestry is defined as the integration of trees and shrubs into crop and animal farming systems to create environmental, economic, and social benefits (USDA, 2011).

Agroforestry is an interdisciplinary approach to land use, different from sum of agriculture and forestry (Lundgren and Raintree, 1983). In Canada, agroforestry is intensively followed in Ontario, Quebec, Saskatchewan and Alberta to increase carbon storage and to reduce greenhouse emission (Baah-Acheamfour et al., 2017). In boreal regions, agroforestry with the utilization of short-rotation of willow plantation was encouraged (McCaughey, 1986). Agroforestry in Northern America mainly consist of tree, forb and shrubs species (Bandolin and Fisher, 1991). In US, the average annual loss of top soil was up to 12 tones in 1976 whereas the annual fertilizer (N-P-K) losses exceeded 50 million tones. The solution for this problem was

multifaceted and included the development of alternative, less energy-intensive technologies, improved soil practices, more efficient and diversified farming system. One such technology solution to this problem lies in the field of agroforestry (Pimentel et al., 1976).

Most of the Newfoundland farms are naturally in an nearly agroforestry like setup consisting of relatively small arable patches surrounded by trees, shrubs and pastures as shown in the **Error! Reference source not found,** 1. In my study field, the agricultural land is surrounded by natural vegetation. It was used for agriculture for more than 50 years expected to have uniformity in terms of nutrient content and microbial diversity in the managed land. On other hand, it is expected to be altered due to management such as tillage and fertilizer application as it directly and indirectly affecting the soil physical, chemical and biological properties of soil compared to natural land. Thus, it is clearly important to investigate the nutrient transfer and microbial diversity and function from agricultural to natural lands and vice -versa.

Delete



Figure 1.1: Farmland setup in Newfoundland; inadvertent Agroforestry?



## 2 Introduction:

### 2.1 *Newfoundland: unique scenario of land-use change in a boreal system?*

Soil types in western Newfoundland especially in Cormack and Deer Lake areas are Orthic Humo-Ferric Podzol and Gleyed Humo-Ferric Podzol respectively (Button, 1983). In Cormack, the texture is loam to sandy loam (well-drained) from the shale and soft sandstone parent material whereas in Deer Lake, it is sandy loam (Imperfectly drained) from granite parent material (Button, 1983). Farming is of minor importance to the economy of Newfoundland because of the poor soil and short growing season. Agriculture in Newfoundland is limited to the areas of south St. John's, near Deer Lake and in Codroy Valley. The major crops grown in these areas are potatoes, turnips, carrots, beets, cabbage and broccoli (Ricketts, 2004).

Under the current impetus for food security in Newfoundland and Labrador boreal forests are currently being converted to agricultural land [64,000 ha over the next 4-5 years] (Agrifoods, 2017). This conversion will affect the physical, chemical and biological properties of soil which in turn affects the soil quality (Costanza et al., 1998). Biotic parameters are expected to be affected and to reflect these changes.

### 2.2 *Hypothesis*

Land use changes from boreal forest to agriculture are expected to affect the physical, chemical and biological parameters of the soil. The act of converting a podzol has drastic effects on the soil structure, and affects soil hydrology (Altdorff et al., 2017). Changes and/or reduces the SOM; eventually longer-term agriculture, especially as associated with livestock farming, leads to an increased addition of manure and plant residues which affects soil carbon cycling and eventually control soil biological functions. Drainage modifies the hydrologic equilibrium effectively modifying the abiotic pressures on the soil microbes (Altdorff et al., 2017).

It is hypothesized therefore that agricultural use of boreal podzols affects the soil abiotic parameters and this is reflected in the microbial taxonomic and functional diversity, and that the relationship between managed and the surrounding non-managed lands will affect the extent of shift in soil biotic parameters:

- Agricultural management of boreal podzols will affect soil microbial taxonomic and functional diversity.

- The shift in taxonomic and functional diversity is directly related to the distance from the non-managed/natural areas.

### 3 Methodology

#### 3.1 Site Description

The study was conducted in Cormack (49°20'40° N 57°19'26°W) in Central Newfoundland, Canada. It has a temperate Boreal climate. Annual average temperature ranges from -1.18°C to 8.06° C and precipitation of 1254 mm of which 332 mm as snow. The soil was classified as Orthic Humo-Ferric Podzol I (Button, 1983). This land is being used for agriculture for 60-70 years. Variable rotation is going on, but mostly entailing wheat, potato and more recently, occasionally corn silage. This field receives manure about twice a year, somewhat irregularly. The field is tile-drained, but the consistency of the drainage efficiency is unknown.

#### 3.2 Site Description and Sampling Design

Sampling was carried out on transects across the field edges, designed to include a managed, tilled agricultural field (*AgField*) and the surrounding, non-tilled, natural field (*NatField*). The field was chosen as a representative of the varied conditions found in the agricultural regions of western Newfoundland due to its variability in the conditions in the *NatField* edge strips **Error!**  
**Reference source not found**,1.

Delet

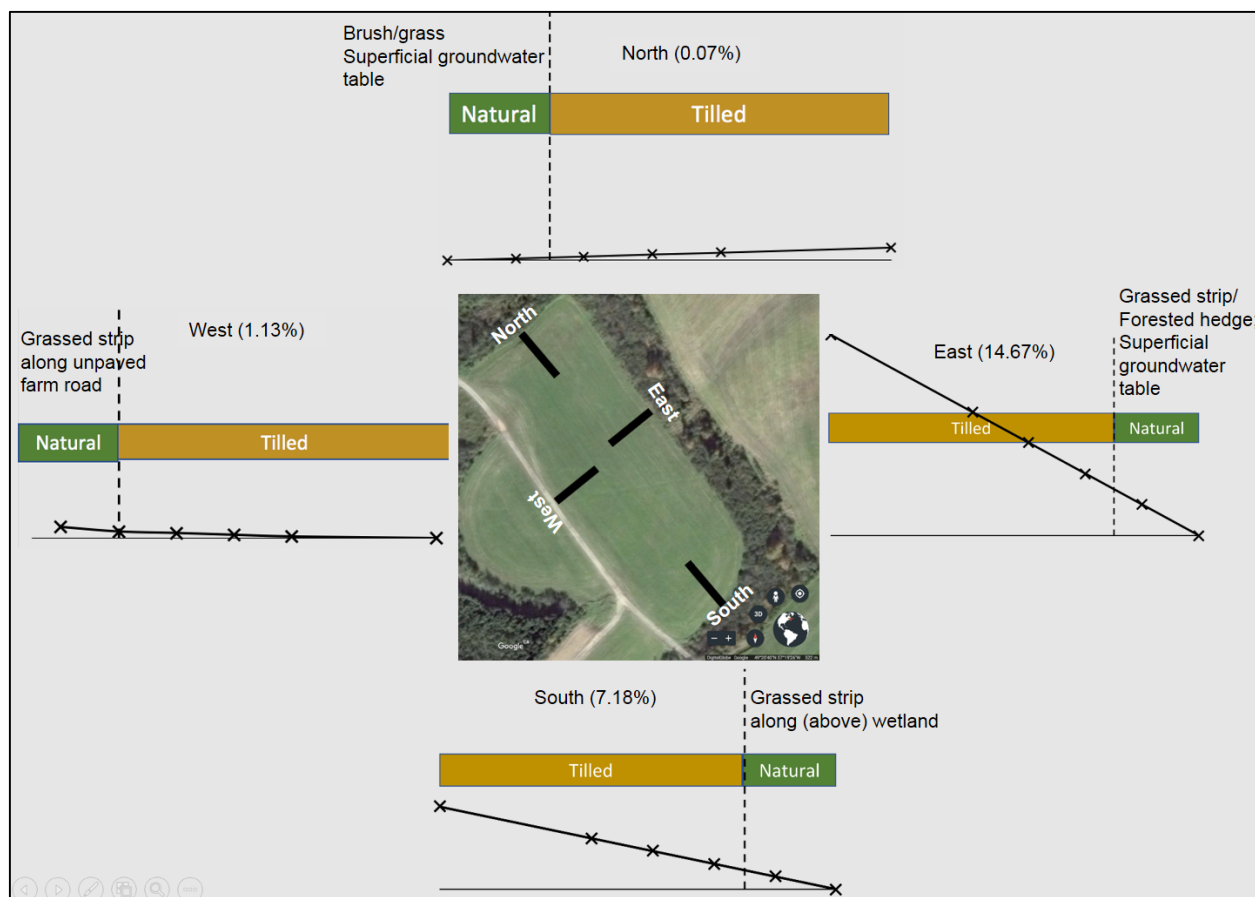


Figure 3.1: Description of sampling transects across the field edge.

Soil sampling was done as a fully replicated design. Sampling was carried out on a transect at the four sites as North (N), East (E), South (S) and West (W) (Figure 3.1). On each transect, two sampling points were located outside the *AgField* (-3 m and -1 m from field edge) and four points inside the *AgField* (1 m, 3 m, 5 m and 10 m) from the field boundary. At each sampling point, soil samples were collected from two depths, identified as Depth 1 (0-10 cm) and Depth 2 (10-20 cm). At each site, each transect was replicated three times (i.e. transect A, B and C; **Error! Reference source not found.**). Thus, every tested condition was run three times, for a total of 144 soil samples (**Table 3.1**; 1). Sampling was done in late August 2015 at grain filling stage of wheat.

Table 3.1: Summary of the sampling design (n=144)

Location	Replicates/Transects (i.e. runs)	Sample location on transects (m)	Sampling depths (cm)
North, East, South, West	A, B, C	-3 (outside the field), -1 (outside the field), 1 (inside the field), 3 (inside the field), 5 (inside the field), 10 (inside the field)	D1, Depth 1 (0-10) D2, Depth 2 (10-20)

The north site has a slope of 0.07% towards the *AgField*. The *NatField* strip consists of mainly shrub/grass. The water table at the outermost sampling point along the sampling gradient was at 0.38 m.

The east site has a slope of 14.67% sloping away from the *AgField*. The *NatField* strip is mainly covered by a mixture of grasses and with a treed region, between the experimental field and a forage field, on the farther east edge of the hedge. The trees are in a surface drainage collection canal, at the outer edge (3-7 m from the edge of the *AgField*). The water table at the outermost sampling point along the sampling gradient was at 0.75 m.

The south site has a slope of 7.2%, sloping away from the *AgField*. The *NatField* is covered by a mix of grasses over a variable width of about 5-10 m, ending into a wetland that is created and collects the tile-drainage from the field. The groundwater table is below 2 m (i.e. a test profile of 2 m depth at the site did not reach the water table).

The west site has a slope of 1.13% sloping away from the *AgField*. The *NatField* strip along the outer edge of the field is a grassed strip of about 2 m width separating the *AgField* from an unpaved farm road.

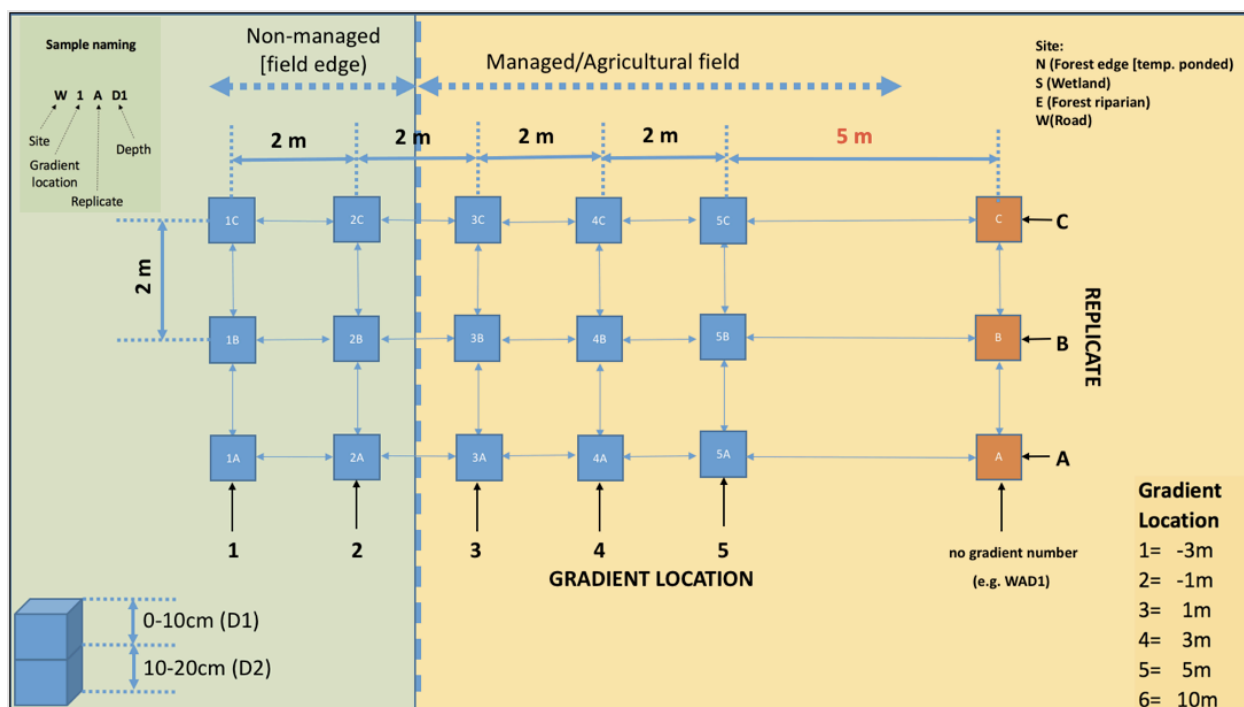


Figure 3.2: Soil sampling design

### 3.3 Sample analysis

#### 3.3.1 Soil handling

For microbial analysis, fresh sample (i.e. within 24 h of sampling) was used to extract DNA. The samples were stored at  $-20^{\circ}\text{C}$ . For each experiment, the frozen samples were thawed for 24-48 h at  $4^{\circ}\text{C}$  and then the required amount of soil was air-dried at room temperature and sieved through 2 mm for moisture content, texture, pH, available P and total cations analyses and through 500  $\mu\text{m}$  for total carbon and nitrogen analyses. To determine moisture content, 10 g of soil sample was oven-dried at  $105^{\circ}\text{C}$  for 24 h. overnight.

#### 3.3.2 Soil Physicochemical Analyses

##### 3.3.2.1 Soil texture

The procedure was carried out as described (M.R. Carter and E.G. Gregorich, 2006). A 50 g of air dried and sieved through 2 mm soil sample was placed in a commercial glass blender (Waring®, Torrington, CT, USA) with 50 mL of Calgon solution ( $50 \text{ mg L}^{-1}$ ) and 350 mL of deionized (DI) water and blended for 5 mins at slow speed. The soil suspensions were poured

into 1 L sedimentation cylinders and the solution was made up to 1 L with DI water. The blank/reference solution was made with 50 mL Calgon solution and 950 mL of DI water in a separate sedimentation cylinder. The soil suspensions and the blank, in the sedimentation cylinders, were stirred vigorously with a wooden plunger for 2 min (about 25 strokes) from close to the bottom to top of the suspension carefully. The Buoyocous hydrometer (Fisher Scientific™, Pittsburgh, PA, USA) was then lowered in the suspension 40 sec after stirring and a reading ( $R_{40}$ ) was taken. After cleaning of the hydrometer, the same procedure was done for the blank solution ( $R_L$ ). After 2 h, the readings ( $R_2$ ) were repeated. Temperature of the solutions was also measured after each reading.

The temperature changes were corrected by adding 0.36 graduation for every 1°C above 20°C and 0.36 graduation was subtracted for every 1°C below 20°C.

Textural proportions were calculated and expressed per air-dry soil mass.

$$\text{Sand \%} = 100 - (R_{40s} - R_L) \times \frac{100}{\text{oven} - \text{dried soil (g)}}$$

$$\text{Clay \%} = (R_{2h} - R_L) \times \frac{100}{\text{oven} - \text{dried soil (g)}}$$

$$\text{Silt \%} = 100 - (\text{Sand \%} + \text{Clay \%})$$

### 3.3.2.2 *pH*

The procedure was carried out as described (M.R. Carter and E.G. Gregorich, 2006).

Ten grams of 2 mm sieved air-dried soil samples were mixed with 20 mL of 0.01M  $\text{CaCl}_2$ . The samples were mixed for 30 secs and let to stand for 1 h. Measurements were taken after 1 h using pH meter (Oakton bench 700 and 2700 series®, Vernon Hills, IL, USA).

A three-point calibration of the pH-meter (at pH 4, 7 and 10) was carried out daily (at 25°C). To ensure quality control, the pH of the 0.01M  $\text{CaCl}_2$  was verified to be in the range of 5.5-6.5 pH and its electrical conductivity (EC) around 2.3  $\text{mS cm}^{-1}$ , at 25°C.

### 3.3.2.3 *Available phosphorus*

Phosphate phosphorus equivalents ( $\text{PO}_4^{3-}\text{-P}$ ) was measured on pre-treated sample via the Mehlich3 standard method (Mehlich, 1984). Soil extraction was done on 2 g of 2 mm sieved air-

dried soil. Soil was added to 50 mL Erlenmeyer flasks and 20 mL of the Mehlich3 extraction solution (0.2 M  $\text{CH}_3\text{COOH}$ , 0.25 M  $\text{NH}_4\text{NO}_3$ , 0.015 M,  $\text{NH}_4\text{F}$ , 0.013 M  $\text{HNO}_3$ , 0.001 M EDTA  $[(\text{HOOCCH}_2)_2\text{NCH}_2\text{CH}_2\text{N}(\text{CH}_2\text{COOH})_2]$ ) was added. The mixture was shaken at 200 rpm for 5 min at room temperature (24 to 27 °C). The mixture was then filtered through a Whatman No.42 filter paper. As the filtrate was not clear, a second filtration was carried out and the filtrate stored at 4 °C.

Filtrates were diluted 20x and analyzed colorimetrically on an AA3 (Seal Auto Analyzer, Fareham Hampshire, United Kingdom). A calibration curve was prepared using 500, 400, 300, 200, 100 and 0 ppm of potassium dihydrogen phosphate solutions. Method blank was used to verify for any cross-contamination. Calibration curve was linear with a correlation coefficient (i.e. regression fit,  $R^2$ ) of 0.9996 to 0.9999.

#### 3.3.2.4 Survey of total cations

Cationic trace elements were analysed via ICP-MS, Inductively Coupled Plasma-Mass Spectrometry (Thermo scientific™ Burlington, ON, Canada): magnesium (Mg), aluminium (Al), iron (Fe), sodium (Na), phosphorous (P), potassium (K), calcium (Ca), manganese (Mn), zinc (Zn), and copper (Cu).

#### *Digestion Procedure:*

Digestion was carried out according to EPA 3050b method (Acid digestion of sediments, sludge, and soils)(USEPA, 1996).

- An aliquot of 0.5 g of 2 mm sieved air-dried homogeneous soil samples was placed in a 50 mL Teflon tube; to this 10 mL of 1:1 trace element grade  $\text{HNO}_3$  was added and the slurry mix covered with a watch glass.
- Samples were then heated at  $95^\circ\text{C} \pm 5^\circ\text{C}$  (name the equipment used here). The temperature was monitored by using a sensor immersed in one of the sample.
- Samples were allowed to cool off for 5 min and supplemented with 5 mL of 70%  $\text{HNO}_3$  solution, and reheated to  $95^\circ\text{C} \pm 5^\circ\text{C}$  and reflux for 5 min.
- Samples were then allowed to cool
- Added 2 mL of deionized (DI) water and 3 mL of 30% hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) solution



- Warming to approximately 60-70 °C
- 1 mL of 30% H<sub>2</sub>O<sub>2</sub> solution was dripped into the slurry mix until no effervescence was observed (not to exceed 10 mL).
- After the sample cooled off the volume was completed to 50 mL by adding DI water.
- The mixture was filtered through Whatman No. 41 paper filter
- Filtrates were stored at 4 °C until analyzed.

Note: The material used for digestion, measuring and preparation of solution was plastic and Teflon which was acid-washed overnight. For every batch of digestion, a blank was used to insure minimal contamination. The Operational conditions for plasma and Instrument Detection Limit, IDL for all cations based on seven replicates are attached in the (see Appendix **Error! Reference source not found.**).

Delet

#### 3.3.2.4.1 Sample Preparation and analysis on ICP-MS:

Analysis for Na, P, K, Ca, Mn, Zn and Cu was done on a 100x dilution with 2% solution of trace element grade nitric acid supplemented with 50 ppb of rhodium as internal standard. For Mg, Al, and Fe a 1000x dilution with 2% solution of trace element grade nitric acid supplemented with 50 ppb of Rhodium as internal standard was used.

Data integrity, instrument performance, method accuracy was ensured by using a Soil Reference Material (SRM): 2711a Montana Soil II, obtained from the National Institute of Standards and Technology (NIST). The SRM was a moderately contaminated soil. Method blanks were used to ensure minimal contamination (Mackey et al., 2010). The comparison of certified values of NIST and obtained values of all cations for NIST is attached in the (see Appendix **Error! Reference source not found.**).

Delet

#### Calibration/Standard Curve:

Calibration curve was obtained using working standards of 0, 10, 50, 100, 200 and 300 ppb, each containing 50ppb of the internal standards (Rh). The working standard stock contained 10 µL mL<sup>-1</sup> of each of Al, Ca, Cu, Fe, K, Mg, Mn, Na, P and Zn. Calibration curves for all runs and isotopes selection are attached in the (see Appendix **Error! Reference source not found.**).

Delet

Table 3.2: Calibration/Standard Curve of standard at different concentration

Standard Concentration (ppb)	Na (ppb) ± 2	P (ppb) ± 2	K (ppb) ± 2	Ca (ppb) ± 2	Mn (ppb) ± 2	Cu (ppb) ± 2	Zn (ppb) ± 2	Al (ppb)	Mg (ppb)	Fe (ppb)
Blank	0	0	0	0	0	0	0	0	0	0
10	95.30	12.98	11.55	15.66	9.57	15.46	6.5	12.48	10.46	12.38
50	117.43	47.71	55.07	61.85	48.35	77.6	45.77	49.56	50.17	51.33
100	147.24	108.06	97.63	104.32	96.92	98.91	96.09	101.37	101.71	103.51
200	208.54	202.44	201.70	208.09	201.97	203.95	202.82	196.51	199.10	198.03
300	264.48	295.97	298.76	290.67	190	292.95	300.24	301.94	299.98	299.84

### 3.3.2.5 Total Carbon and Nitrogen:

Total carbon and nitrogen, were analyzed on a Perkin Elmer model 2400 CHNS/O Series II (100V) elemental Analyzer (Waltham, Massachusetts, USA)(PerkinElmer, 2011). This method uses combustion and reduction columns at 925 °C and 640 °C respectively. Approximately 13-15 mg of 500 µm sieved air-dried soil samples were weighed in a tin capsule (8x5mm).

Calibration was done using 1.25 to 2.50 mg of Acetanilide Standard.

Accurately weighed Acetanilide standard was run to obtain a conversion factor of each element (%C, %H, %N) from the detector. By normalizing detector reading by weight and dividing the normalized reading by the theoretical %weight of C, H and N in the standard. These values are called K-Factor, which is automatically given by machine (Veysey, 2015).

Table 3.3: Minimum Precision Criteria for Blanks and K-Factors with reproducibility

% Elements	Acetanilide as sample	Acetanilide as K-Factor	Blank
Carbon	71.09 +/- 0.40	16.5 +/- 3.5	<100 +/- 30
Hydrogen	6.71 +/- 0.40	50.0 +/- 20.0	200-300+/- 100
Nitrogen	10.36 +/- 0.40	6.0 +/- 3.0	<50+/- 16

### 3.3.3 Microbiological methods

Microorganisms act as an indicator of soil health, which cannot be assessed only from physical

or chemical parameters of soil or by analyzing higher organism level. Microorganisms have quick response to changes in environment and will survive only under favorable condition. Therefore, analyzing the microbial community and their function helps us to find the quality of soil/soil health. Sometimes changes in the microbial community have noticeable change in soil physical and chemical parameters (Kibblewhite et al., 2008; Zornoza et al., 2009).

### *3.3.3.1 DNA extraction*

DNA was extracted using a Power Soil DNA isolation kit (MO BIO lab, QIAGEN Inc, Carlsbad, CA, USA) from ~0.25 g of fresh soil per extraction reaction. Extraction steps were carried out according to manufacturer's protocol (MO BIO lab, QIAGEN Inc, Carlsbad, CA, USA). After extraction, a 1.00 µL aliquot was used to verify for DNA yield and quality on a Nano Drop 2000c spectrophotometer (Thermo scientific™ Burlington, ON, Canada) at the wave lengths of 260 and 280 nm. The 260/280 nm ratio is commonly employed to verify the efficiency of protein removal during extraction, and thus purity of DNA extract.

### *3.3.3.2 Sequencing*

#### *3.3.3.2.1 Bacterial taxonomic diversity via amplicon sequencing*

Sequencing of selected hypervariable regions of the 16S ribosomal RNA (rRNA) gene sequencing is a common amplicon sequencing protocol employed to describe bacterial taxonomic diversity in environmental samples. The 16S gene is used as a genetic marker because it is a household gene conserved over time, with a relatively constant and predictable mutation rate, as it carries out essential functions, translating of mRNA (messenger RNA) into protein; it is also a relatively short gene, at 1.5 k base pairs (bp), and it is therefore fast and cheap to sequence. Amplicon sequencing for the DNA extracts was carried out on an Illumina MiSeq Sequencing platform at Genome Quebec.

The following barcoded primers were employed:

515F: (5' GTGCCAGCMGCCGCGGTAA 3')

806R: (5' GGACTACHVGGGTWTCTAAT 3')

This produces an amplicon of 253 bp in length, excluding the primers' sequences. This primer set targets the V4 region of both Bacteria and Archaea and has a high sequence coverage, of about 93.6 for bacteria and 90.9 for archaea (Wu et al., 2015).

#### 3.3.3.2.2 *Fungal taxonomic diversity via amplicon sequencing*

Fungal taxonomy was also assessed via amplicon sequencing, targeting the ITS region (Internal Transcribed Spacer) of the rDNA a commonly used barcoding marker for fungi (Porter and Brian Golding, 2011). ITS refers to spacer DNA situated between the small subunit and large subunit of ribosomal RNA (rRNA) in the chromosome (White et al., 1990; Wu et al., 2002).

Primers Used (Wu et al., 2003).

1. Forward, ITS1: 5'-TCCGTAGGTGAACCTGCGG-3'
2. Reverse, ITS4: 5'-TCCTCCGCTTATTGATATGC-3'

#### 3.3.3.3 *Sequence Analysis:*

*16SrRNA* and ITS sequencing was done Illumina MiSeq Sequencer. The generated sequences were then processed through QIIME (Quantitative Insights Into Microbial Ecology) pipeline which has 95 cut off, which performs demultiplexing, removal of primers, quality based sequence trimming, obtaining Operational Taxonomic Unit (OUT) table and assemblage of paired-end sequences. Chimeras (two or more biological sequences joined incorrectly) was checked using UCHIME.

Profiling microbial community via High-throughput sequencing (in this study, we used Illumina) is an emerging powerful tool. MiSeq produces a data of > 1 gigabase (Gb) via sequence by synthesis technique which has quality comparable to HiSeq® 2000 platform. In this process, the reads/sequences were scanned for contaminants and PhiX reads (0-25% of the samples are contaminated with PhiX). From the above process of screening, one read pair was lost and was discarded. The remaining pairs were trimmed to 165bp by default depending on the quality on sequence run and amplicon length. These pairs were assembled to reconstruct 16S amplicons using FLASH software. The assembled reads were then screened for quality score of >10 to <30 nucleotides, Ns. These reads were referred as filtered reads. These filtered reads were then clustered using in-house clustering algorithm. In this process, 100 identity reads were clustered then at 99 identity. Clusters with abundance <3 were eliminated. The remaining clusters were scanned for chimeras using UCHIME denovo and UCHIME reference and clustered at 97 identity, which final clusters are called operational taxonomic unit (OTUs). OTUs were then analyzed for taxonomic distribution using a combination of in-house program and scripts from QIIME software. In this process, OTUs were classified with RDP classifier using an in-house program containing a complete set of Greengenes database supplemented with eukaryotic

sequences from the Sliva databases and a customized set of mitochondria and chloroplast 16S reads. ITS database consists of the UNITE ITS database (ITS1-2) region. The RDP classifier gave a score of 0-1 to each taxonomic level of each OTUs and the taxonomic level having a score  $\geq 0.5$  was left for reconstruction of final lineage. From the taxonomic lineage, a raw OTU table was generated; from there an OTU table of Bacteria and Fungi was generated. Then it was checked for rarefaction and a final OTU table was generated.

Microbial community functions cannot be studied directly by profiling phylogenetic marker genes such as the 16s rRNA. PICRUSt (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States), a computational approach was used in this study to predict the metagenomic functional composition using 16S rRNA and Greengene database.

#### *3.3.3.4 Taxonomic diversity*

##### *3.3.3.4.1 Bacteria*

There were a total of 22,144,080 bacterial read sequences obtained, of which 3,192,399 sequences were of insufficient quality; the remaining 18,951,681 reads were assembled in to 8,774,064 reads, of which 6,889,880 passed the QC (Quality Control) protocols as implemented at the sequencing facility (GenomeQuebec) in the standard QIIME pipeline. These were clustered into 3,280,215 sequences using a 97% identity cut-off. Eventually 3,267,545 sequences were clustered into 18,763 OTUs (Operational Taxonomic Units).

##### *3.3.3.4.2 Fungi:*

There were a total of 25,909,070 fungal read sequences, of which 1,054,075 sequences were of insufficient quality; the remaining 24,854,995 reads were assembled in to 9,791,260 reads, of which 6,578,766 were passed the QC (Quality Control) protocols as implemented at the sequencing facility (GenomeQuebec) in the standard QIIME pipeline. These were clustered into 6,360,925 sequences using a 97% identity cut-off. Eventually 6,359,109 sequences were clustered into 3,932 OTUs (Operational Taxonomic Units).

#### *3.4 Statistical Analysis*

##### *3.4.1 Physicochemical Analyses*

The significance of the replicates and factors on the physical and chemical soil parameters, and the significance of the relevant variables, *i.e.* statistical covariates, were determined using a

general linear model (GLM);  $p$  values  $\leq 0.05$  were used to determine statistical significance (Shin, 1995). These tests were carried out in Minitab. Multivariate analyses were carried out by Principal Component Analysis (PCA) using the Orange vs. 3.8 software (Mining, 2016; Orange.biolab, 2014).

### 3.4.2 Microbial Taxonomic Diversity Analysis

The clustered OTUs were classified taxonomically by alignment against the Greengene database. Note that the approach is only accurate up to the genus level. Bacterial OTUs dataset was first depleted of the mitochondrial, cyanobacterial/chloroplast and archaeal sequences. The success of this classification approach is summarized in Table 6.

Table 3.4: Summary of the taxonomic assignment success

<b><u>Bacteria</u></b>		OTUs (sequence count)
Total		18,763
After Deleting Mitochondria, Cyanobacteria and Archaea		17,821 (25,07,590)
<b><u>Fungi</u></b>		
Total		3,932 (46,41,274)
<b><u>Unclassified Sequences:</u></b>		
Taxonomic Level	Bacteria OTUs (sequence count)	Fungal OTUs (sequence count)
Phylum	75, (2,151)	785, (80,890)
Class	111, (4,545)	1,189, (2,26,000)
Order	180, (6,287)	1,569, (3,11,370)
Family	261, (9,461)	1,777, (6,30,310)
Genus	362, (20,330)	1,947, (7,85,419)

The data for the NBD2 sample was eventually eliminated from all bacterial analyses due to its unsatisfactory rarefaction curve.

#### 3.4.2.1 Alpha Diversity

Alpha diversity, *i.e.* the diversity within each sample, was assessed for both bacteria and fungi at OTUs level; this included Simpson 1-D, Dominance-D (number of individuals belonging to the most abundant species), Shannon-H, and Chao1 indices. Tests were carried out in the PAST3 ecological statistics software (Hammer 2017).

Dominance= 1-Simpson,  $D = \text{Sum}\left(\left(\frac{n_i}{n}\right)^2\right)$

Where  $n_i$  is the number of individuals of taxa

$n$  is the total number of individuals

Simpson1-D (diversity based on abundance and richness),

Simpson Index=1-Dominance

Shannon H (diversity based on abundance and richness)

$$H = \text{sum} \left[ \frac{n_i/n}{n(n_i/n)} \right]$$

Chao-1 (Calculates diversity with accounting of rare species) (Hammer, 2017).

Singletons and doubletons were not removed for this analysis, and all analyses were performed at the OTU level. Approximate confidence intervals were computed using a bootstrap with 9999 cycles.

#### 3.4.2.2 Beta Diversity

Analyses for beta diversity, *i.e.* the similarity and dissimilarity levels between samples, of the bacterial and fungal communities were carried out at the class taxonomic level using EstimateS Mac910 Statistical software (Colwell and Elsensohn, 2014); a Bray-Curtis similarity matrix was employed. Before this analysis, the datasets were depleted of OTUs represented only by singletons or doubletons.

A SIMPER analysis (Similarity Percentage) was carried out at the class taxonomic level. SIMPER is another assessment of the beta diversity that calculates the proportional contribution of each variable (class proportional abundance in this case) to the similarity or dissimilarity between treatments. The proportional contribution to dissimilarity of bacteria or fungi was carried out at class level. SIMPER was carried out using the PAST3 ecological statistics software (Hammer 2017) using a Bray-Curtis dissimilarity matrix. For this analysis, singletons and doubletons were not removed.

#### 3.4.2.2.1 *Discriminant analysis*

The capability of predictive approaches to identify a given treatment option, e.g. site or location on the sampled gradient may be employed to identify similarity levels. Neural network (NN), Logistic regression (LR), Naïve Bayesian (NB), and Support Vector Machine (SVM) approaches as implemented in the Orange vs. 3.8 (Demsar et al., 2013) were employed. The first two were found to be most effective and are therefore presented and discussed in the respective sections later in the manuscript. The quality of the predictive analyses is described by several quality indicators:

- Area under an Receiver Operating Characteristic (ROC) curve, (AUC) (Wigton et al., 1986) describes the accuracy of a test, or how well the test separates the group being tested from other groups. An area of 1 represents a perfect prediction; an area of 0.5 represents a meaningless prediction (Demsar et al., 2013).
- The Classification accuracy (CA) is the proportion of correctly classified examples.
- The F score (F1) is the harmonic average of the precision and recall; an F1 score ranges between 0 and 1 with 1 being best (Powers, 2011).
- Precision, is the number of correct positive results divided by the number of all positive results returned by the classifier (Demsar et al., 2013).
- Recall, is the number of correct positive results divided by the number of all samples that should have been identified as positive (Demsar et al., 2013).

For these analyses, we have employed the taxonomic diversity of bacteria or fungi, at OTUs level and also the PICRUST inferred functional diversity. As the latter is dependent on the taxonomic identification of bacteria via 16s rDNA structure it is expected to offer a similar discriminant power. On the other hand, the non-uniform distribution of functions across taxa does lead to the discriminant power of the said inferred functional profile to be sufficiently distinct from the one of the bacterial taxonomic profile.

#### 3.4.3 *Correlation between microbial diversity and soil properties*

An assessment of the correlation between microbial taxonomic diversity and soil physicochemical properties was carried out using Megan bioinformatics software (Huson et al., 2007). For this analysis, the class level taxonomic diversity was used. The 17,710 bacterial



OTUs were, classified into 49 taxonomic classes, whereas the 2,743 fungal OTUs were classified into 23 taxonomic classes. A total of 111 bacterial OTUs and 1189 fungal OTUs could not be classified at class level.

#### *3.4.4 Functional Diversity*

Multivariate analysis of bacterial functional diversity for all metabolic functions (132 functions) except functions relevant to the metabolism of xenobiotics (21 functions), which were assessed separately, was carried out by, Principal Component Analysis (PCA) using the Orange vs. 3.8. software (Mining, 2016; Orange.biolab, 2014). Functional diversity was inferred from the 16s RNA based taxonomic diversity as described in section (Appendix 7.11). An assessment of the relationship between the inferred functional diversity and the soil parameters was also carried out in the same software using Double Hierarchical Dendogram Heat Map.

## 4 Results

### 4.1 Soil Physiochemical Parameters

#### 4.1.1 Soil Physical properties

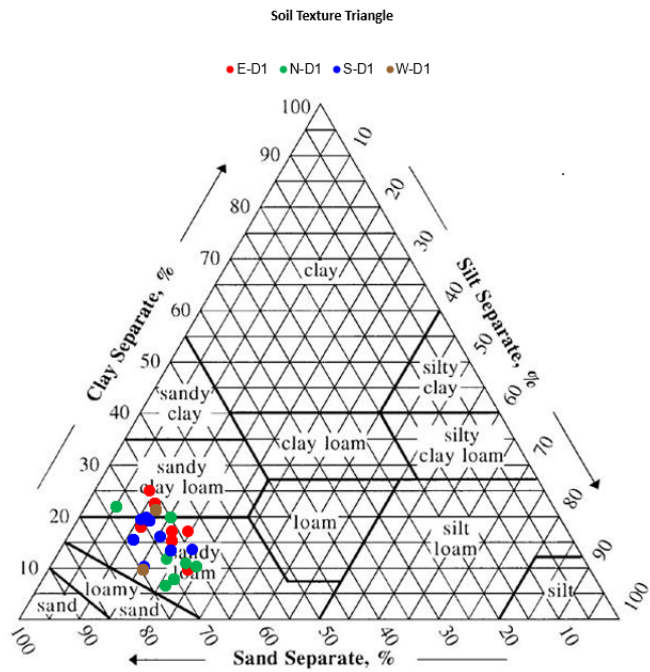
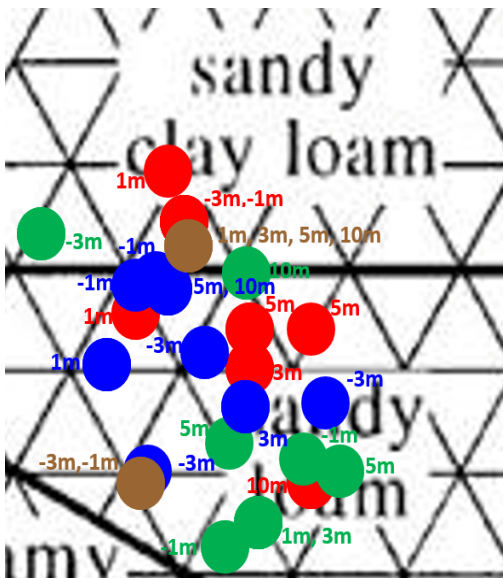
##### Soil Texture

The texture across the entire test site was relatively similar, being classified as sandy loam or sandy clay loam (Figure 4.1). There was a general trend of the lower depth to have a slightly finer texture than the top soil depth.

Statistical analyses were carried out on the proportions of sand (Figure 4.2) and clay (Figure 4.3). Sand% was found to be significantly highest at the west site, with a 31.2% average, and the lowest at the south site with an average of 28.9% irrespective with depth (see Appendix 7.1.1). The proportion of sand is generally higher in the *AgField* samples versus the *NatField*, but not statistically significant (see Figure 4.2, Appendix 7.1.2).

The proportions of clay were found to be higher in East site (33.2%) and significantly lower for the North site (27.9%) (see Appendix 7.2.1). The Depth 2 has significantly more clay (32.7%) than Depth 1 (30.0%) (see Appendix 7.2.3).

## Depth 1



## Depth 2

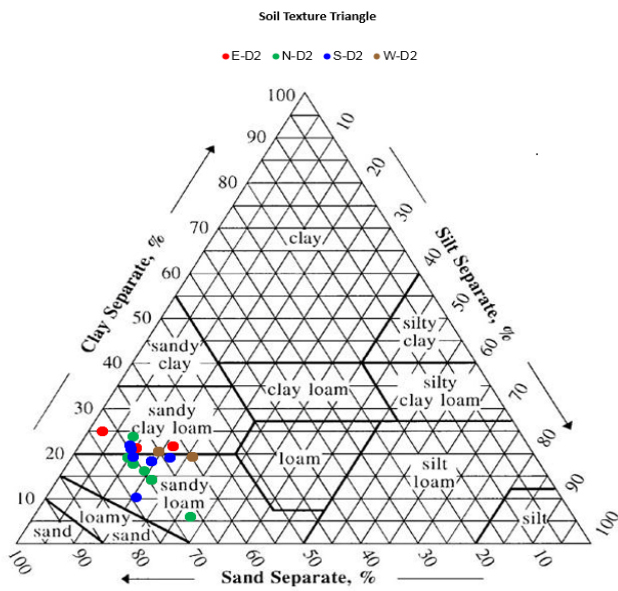
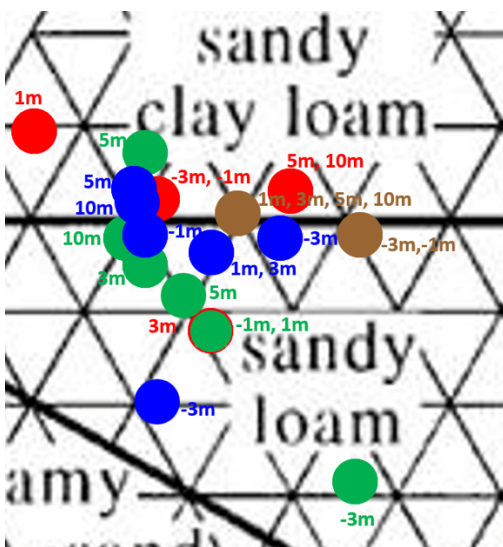


Figure 4.1: Textural distribution of soil samples

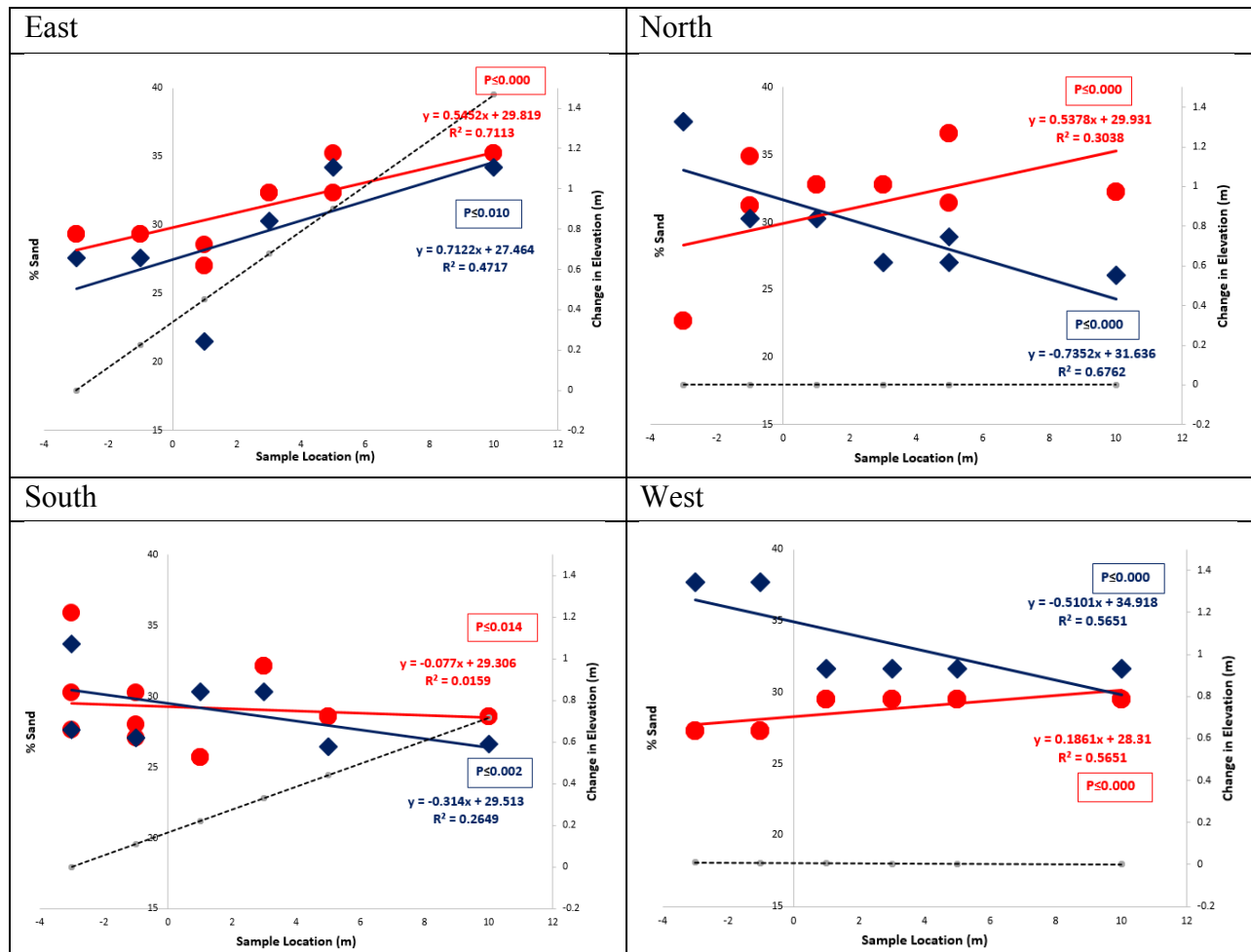


Figure 4.2: Sand% across the sampling gradients in both the depths at all sampling sites

Variation of the sand% for Depth 1 (0-10 cm) and Depth 2 (10-20 cm) with respect to management intensity/sample location (-3 m and -1 m at *NatField* and 1 m, 3 m, 5 m and 10 m in the *AgField*) and slope with the significant difference (ANOVA, General Linear Model,  $P \leq 0.05$ ).  $R^2$  describes the variability in measurements along the length of the gradients (m). The red dots describe Depth 1 data while the blue diamonds describe the Depth 2 data; the dotted lines describe the % topographical slope (secondary y-axis).

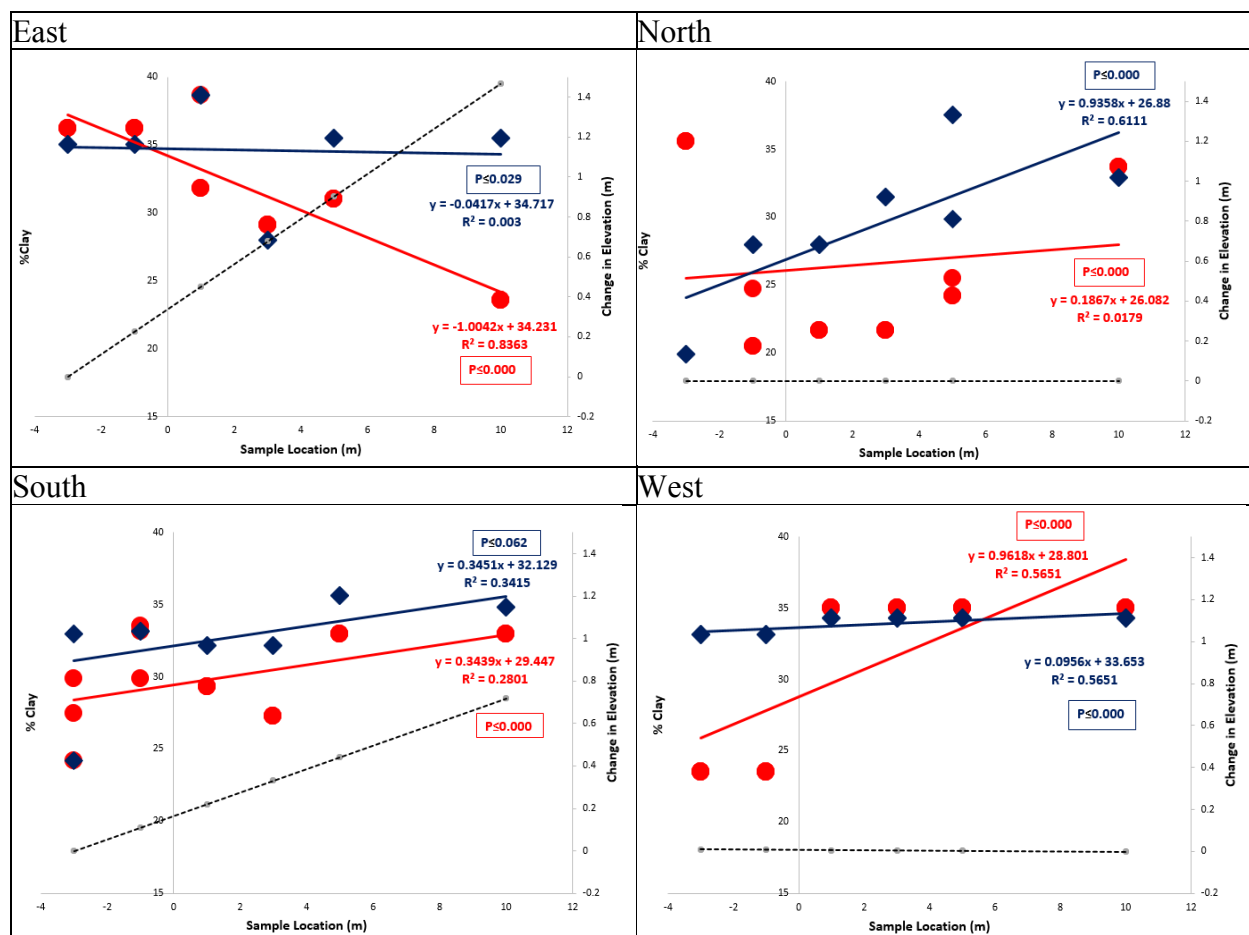


Figure 4.3: Soil texture across the sampling gradients; clay (%) in both the depths at all sampling sites

Variation of the clay% for Depth 1 (0-10 cm) and Depth 2 (10-20 cm) with respect to management intensity/sample location (-3 m and -1 m at *NatField* and 1 m, 3 m, 5 m and 10 m in the *AgField*) and slope with the significant difference (ANOVA, General Linear Model,  $P \leq 0.05$ ).  $R^2$  describes the variability in measurements along the length of the gradients (m). The red dots describe Depth 1 data while the blue diamonds describe the Depth 2 data; the dotted lines describe the % topographical slope (secondary y-axis).

#### 4.1.2 Soil Chemical parameters

##### pH

Soil pH was found to be significantly higher for north site (mean = 6.09) and significantly low for the east (mean of 5.27) (Figure 4.4) (see Appendix 7.3.1). Within the *AgField*, the 10 m gradient location has produced significantly lower pH values (mean of 5.32) than the *NatField*, (e.g. a mean of 5.78 at -1 m gradient location) (see Appendix 7.3.2). The pH of the Depth 1 has an average pH slightly higher than Depth 2 (means of 5.66 vs 5.55) (see Appendix 7.3.3).

##### Sodium

Soil sodium (Na) (Figure 4.5: 4.5) was found to be significantly high in the west site (mean of 318.6 mg/kg soil dry matter [DM]) and significantly low in the South site (mean of 73.7 mg/kg soil DM) (see Appendix 7.4.1). Within the *AgField*, the 5 m gradient location has significantly higher Na (mean of 360.7 mg/kg soil DM) than the -3 m *NatField* gradient location (65.8 mg/kg soil DM) (Figure 4.5: and Appendix 7.4.2).

##### Potassium

Soil potassium (K) was found to be significantly higher in the East and West sites 3957.7 and 3823.7 mg/kg soil DM respectively) and low in the North site (3309.4 mg/kg soil DM) (**Error! Reference source not found.**) (Appendix 7.5.1).

##### Calcium

Soil calcium, Ca is significantly higher in north site with the mean of 4392.7 (mg/kg) than rest of the sites (see Figure 4.7: Appendix 7.6.1). Ca is significantly lower in *AgField*, 10 m and 1 m with the mean of 2553.7 (mg/kg) and 1773.4 (mg/kg), respectively than in *NatField*, -1 m and -3 m with the mean of 3996.6 (mg/kg) and 3696.0 (mg/kg), respectively (see Appendix 7.6.2)

##### Manganese

Soil manganese, Mn is significantly lower in north site with the mean of 527.4 (mg/kg) than rest of the sites (see Figure 4.8: Appendix 7.7.1). *AgField*, 10 m has significantly higher Mn than other sampling location (see Appendix 7.7.2)

##### Copper

Soil copper, Cu is significantly lower in North site with the mean of 40.3 (mg/kg) than rest of the sites (see Figure 4.9, Appendix 7.8.1). *AgField*, 10 m has significantly higher Cu with the mean of 119.3 (mg/kg) than *NatField*, -3 m with the mean of 40.7 (mg/kg) in all the sites (see Appendix 7.8.2).

### *Zinc*

Soil zinc, Zn is significantly lower in North site with the mean of 84.4 (mg/kg) than rest of the sites (see [Figure 4.10](#), Appendix 7.9.1). *AgField*, 1 m has significantly higher Zn with the mean of 128.3 (mg/kg) than *NatField*, -3m and -1m with the mean of 107.3 (mg/kg) and 107.1 (mg/kg) respectively in all the sites (see Appendix 7.9.2)

Delet  
Form

### *Magnesium*

Soil magnesium, Mg is significantly high in the East site with the mean of 18770(mg/kg) and significantly low in West site with the mean of 14312 (mg/kg) (see [Figure 4.11](#), Appendix **Error! Reference source not found.**) *AgField*, 10 m has significantly low Mg with the mean of 14972 (mg/kg) and *NatField*, -1m has high Mg with the mean of 17969 (mg/kg) in all the sites (see Appendix 7.10.2).

Delet  
Form  
Delet

### *Aluminium*

Soil aluminum, Al is significantly high in the East site with the mean of 33663 (mg/kg) and low in the North and South sites with the mean of 28381 (mg/kg) and 28273 (mg/kg) respectively (see [Figure 4.12](#), Appendix 7.11.1)

Form  
Delet

### *Iron*

Soil iron, Fe is significantly higher in the East site with the mean of 48045.60(mg/kg) than rest of the sites (see [Figure 4.13](#), Appendix 7.12.1)

Form  
Delet

### *Total Organic Carbon*

Soil Total Organic Carbon, TOC is significantly high in the South and the North sites with the mean of 44403 (mg/kg) and 44342 (mg/kg) respectively and low in the West and East sites with the mean of 35408 (mg/kg) and 32483 (mg/kg) respectively (see Appendix **Error! Reference source not found.**) *AgField*, 5 m has significantly lower TOC with the mean of 36746 (mg/kg) than the *NatField*, -3 m with the mean of 43983.30 (mg/kg) in all the sites (see Appendix **Error! Reference source not found.**) Depth 1 has significantly higher TOC with the mean of 41999 (mg/kg) than Depth 2 with the mean of 36319 (mg/kg) (see [Figure 4.14](#), Appendix 7.13.3).

Delet

Delet  
Form  
Delet

### *Total phosphorus*

Soil Total Phosphorus, TP is significantly different from each other in all the sites with the mean of 2154 (mg/kg) in the South site and 1623 (mg/kg) in the west site and 1336 (mg/kg) in the East

site and 1118 (mg/kg) in the North site (see Appendix 7.14.1). *AgField*, 1 m and 5 m has significantly higher TP with the mean of 2084 (mg/kg) and 1615 (mg/kg) respectively than the *NatField*, -3 m and -1 m with the mean of 1302 (mg/kg) and 1291 (mg/kg) respectively (see [Figure 4.15](#), Appendix 7.14.2). Depth 1 has significantly higher TP with the mean of 1661 (mg/kg) than Depth 2 with the mean of 1454 (mg/kg) (see Appendix 7.14.3).

#### *Available phosphorus*

Soil available phosphorus,  $\text{PO}_4$  is significantly high in the South and the West sites the mean of 4.10 (mg/kg) and 3.88 (mg/kg) respectively and significantly low in the East and the North sites with the mean of 2.27 (mg/kg) and 2.16 (mg/kg) respectively (see [Figure 4.16](#), Appendix 7.15.1). Depth 1 has significantly higher P with the mean of 3.43 (mg/kg) than Depth 2 with the mean of 2.77 (mg/kg) (see Appendix 7.15.3)

#### *Total nitrogen*

Soil Total Nitrogen, TN is significantly higher in South site with the mean of 3383 (mg/kg) than rest of the sites (see Appendix 7.16.1). Depth 1 has significantly higher P with the mean of 2921 (mg/kg) than Depth 2 with the mean of 2407 (mg/kg) (see [Figure 4.17](#), Appendix 7.16.3).

Delete  
Form

Delete  
Form

Form  
Delete



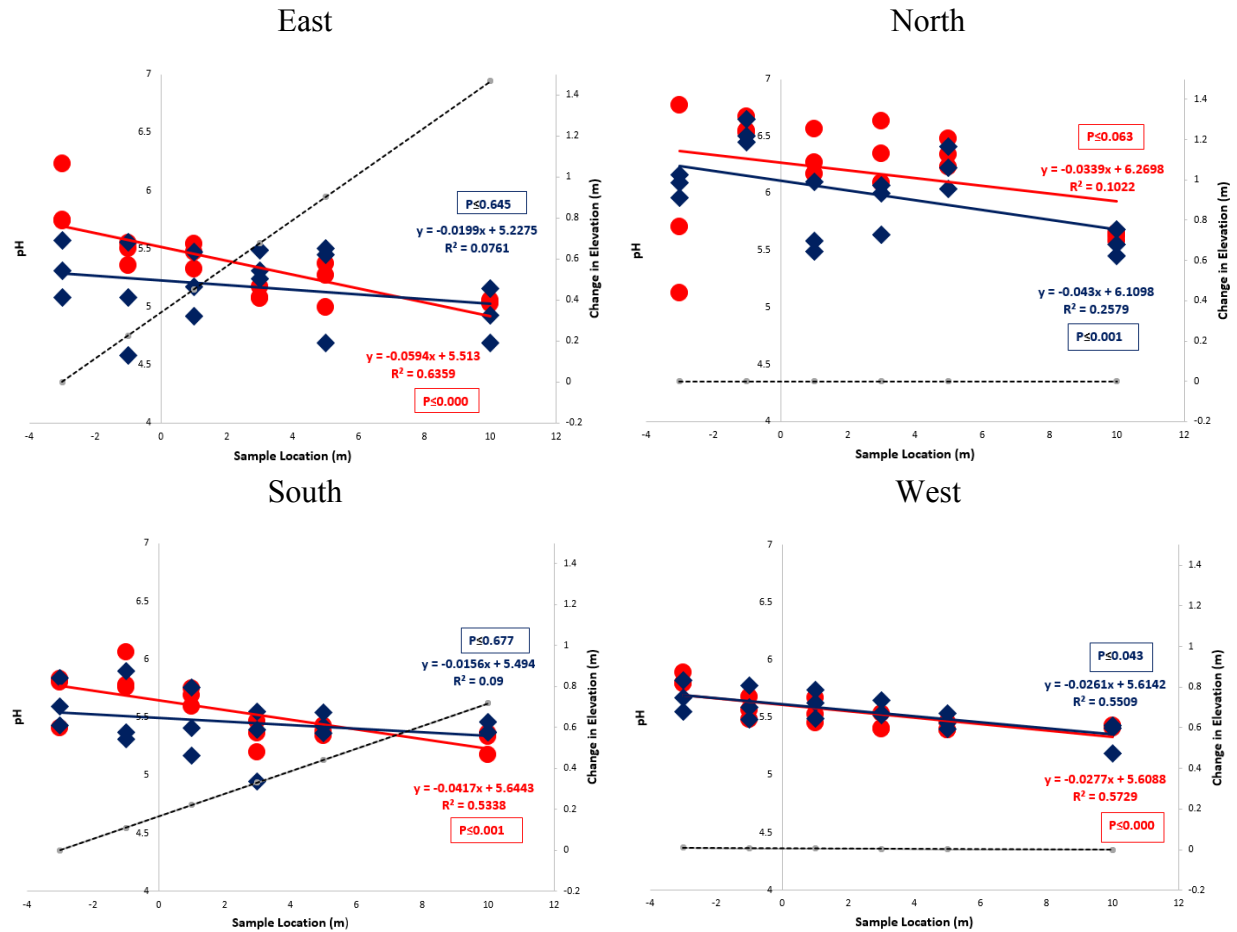


Figure 4.4: Soil pH across the sampling gradients for both depths at all sampling sites

Variation of soil pH measurements for Depth 1 (0-10cm) and Depth 2 (10-20cm) with respect to management intensity/sample location (-3m and -1m at *NatField* and 1m, 3m, 5m and 10m in the *AgField*) and slope with the significant difference (ANOVA, General Linear Model,  $P \leq 0.05$ ). The regression coefficient ( $R^2$ ) describes the variability in measurements along the length of the gradients (m). The red dots describe Depth 1 data while the blue diamonds describe the Depth 2 data; the dotted lines describe the % topographical slope (secondary y-axis).

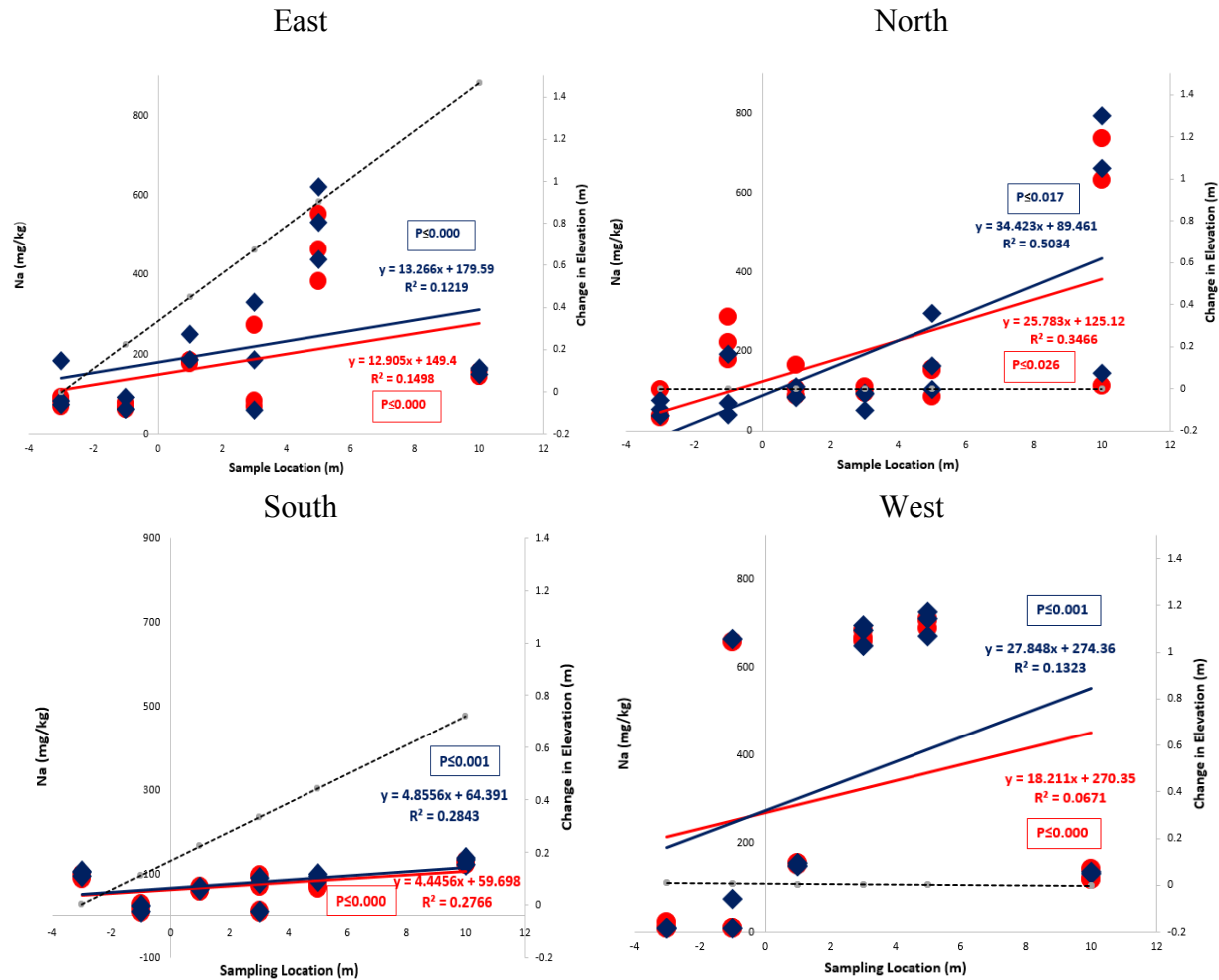


Figure 4.5: Concentration of sodium (Na) in soils across the sampled gradients for both depths at all sampling sites

Variation of Na measurements for Depth 1 (0-10cm) and Depth 2 (10-20cm) with respect to management intensity/sample location (-3m and -1m at *NatField* and 1m, 3m, 5m and 10m in the *AgField*) and slope with the significant difference (ANOVA, General Linear Model,  $P \leq 0.05$ ). The regression coefficient ( $R^2$ ) describes the variability in measurements along the length of the gradients (m). The red dots describe Depth 1 data while the blue diamonds describe the Depth 2 data; the dotted lines describe the % topographical slope (secondary y-axis).

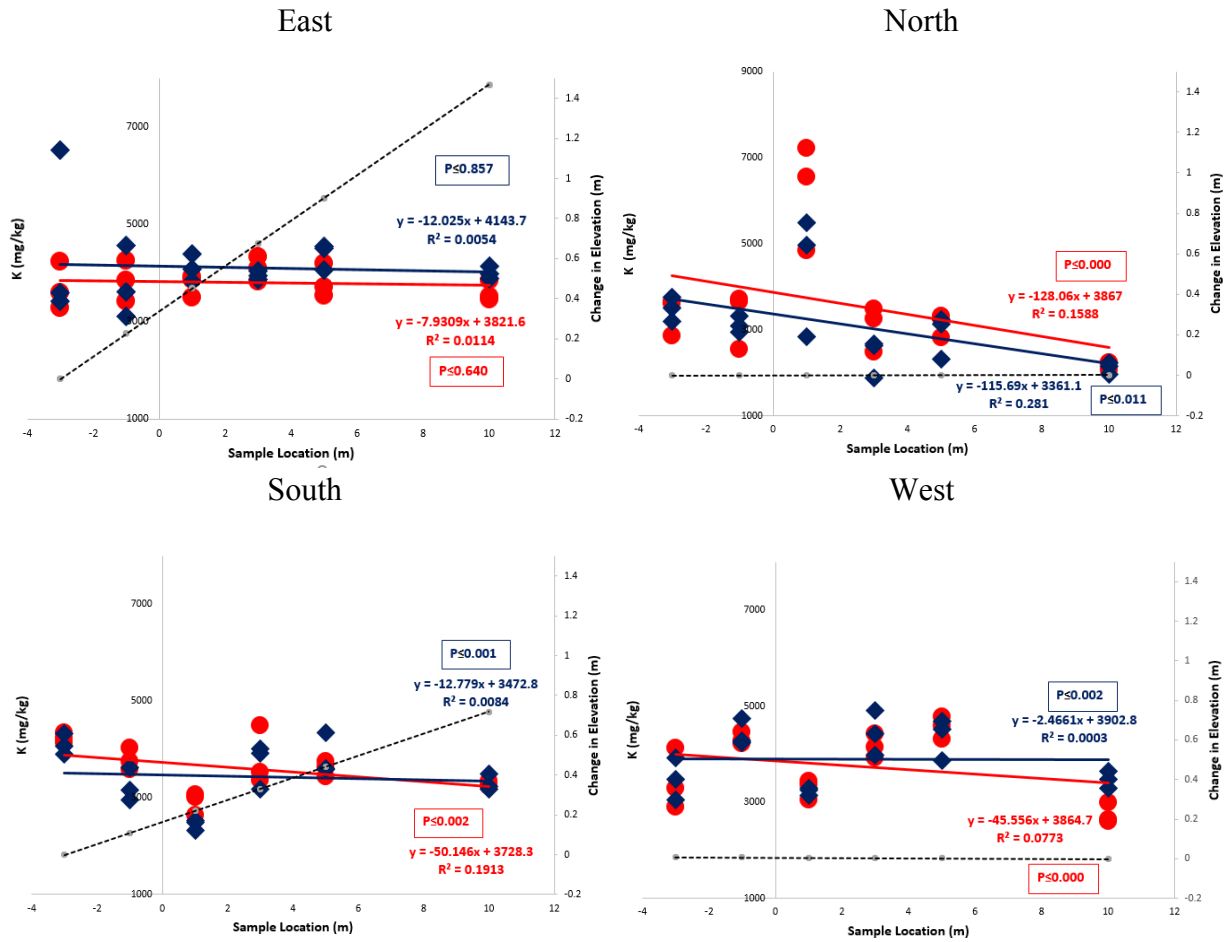


Figure 4.6: Concentration of potassium (K) in soils across the sampled gradients for both depths at all sampling sites

Variation of K measurements for Depth 1 (0-10cm) and Depth 2 (10-20cm) with respect to management intensity/Sample Location (-3m and -1m at *NatField* and 1m, 3m, 5m and 10m in the *AgField*) and slope with the significant difference (ANOVA, General Linear Model,  $P \leq 0.05$ ).  $R^2$  describes the variability in measurements along the length of the gradients (m). The red dots describe Depth 1 data while the blue diamonds describe the Depth 2 data; the dotted lines describe the % topographical slope.

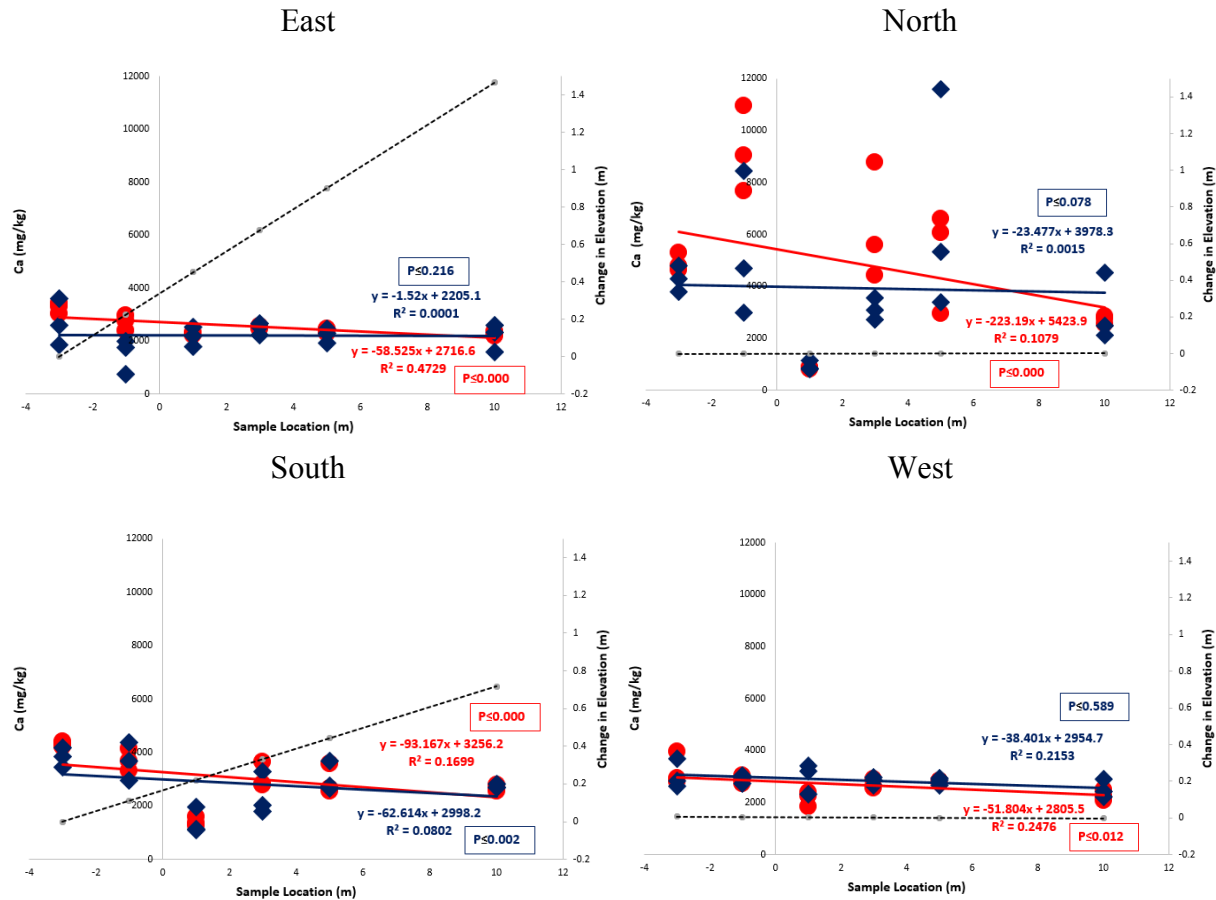


Figure 4.7: Concentration of calcium (Ca) in soils across the sampled gradients for both depths at all sampling sites

Variation of Ca measurements for Depth 1 (0-10cm) and Depth 2 (10-20cm) with respect to management intensity/Sample Location (-3m and -1m at *NatField* and 1m, 3m, 5m and 10m in the *AgField*) and slope with the significant difference (ANOVA, General Linear Model,  $P \leq 0.05$ ).  $R^2$  describes the variability in measurements along the length of the gradients (m). The red dots describe Depth 1 data while the blue diamonds describe the Depth 2 data; the dotted lines describe the % topographical slope.

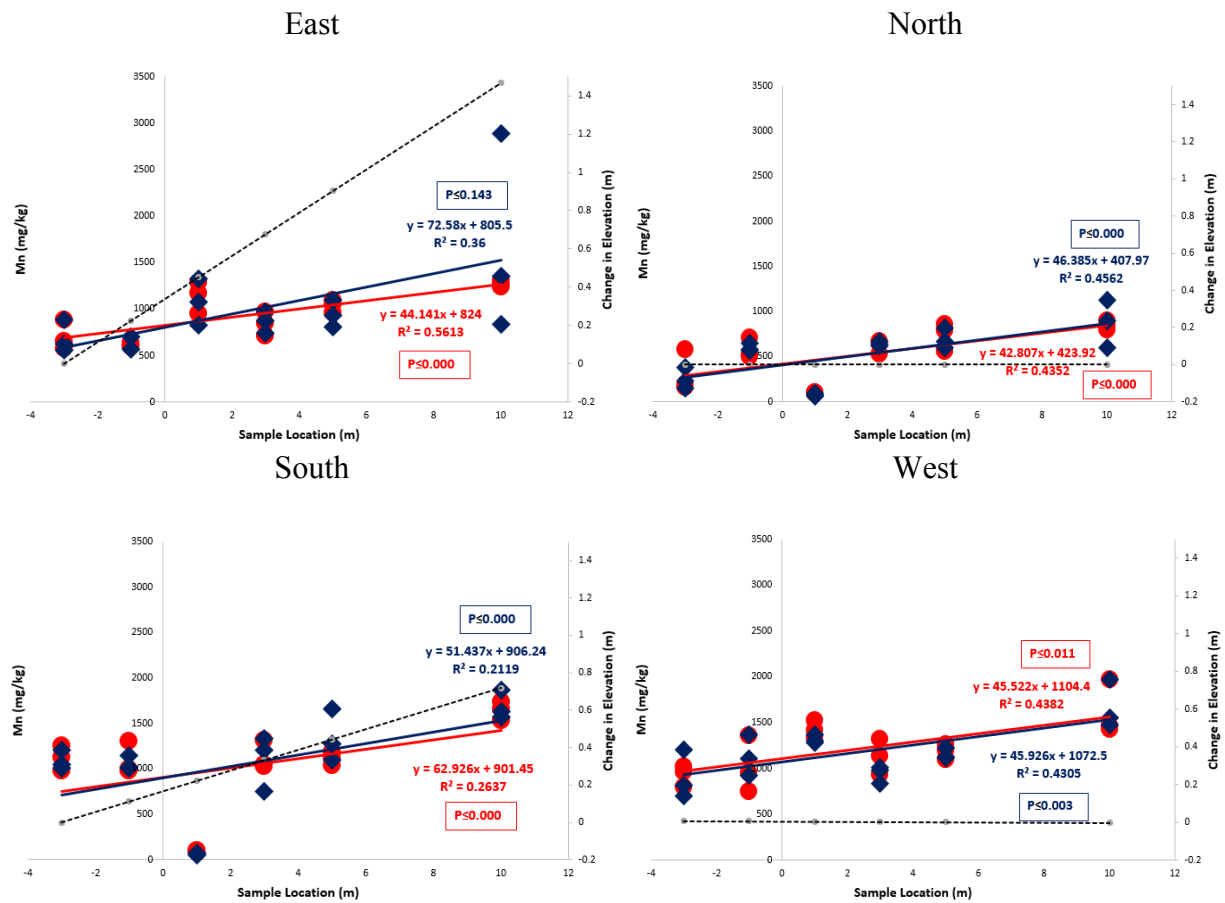


Figure 4.8: Concentration of manganese (Mn) in soils across the sampled gradients for both depths at all sampling sites

Variation of Mn measurements for Depth 1 (0-10cm) and Depth 2 (10-20cm) with respect to management intensity/Sample Location (-3m and -1m at *NatField* and 1m, 3m, 5m and 10m in the *AgField*) and slope with the significant difference (ANOVA, General Linear Model,  $P \leq 0.05$ ).  $R^2$  describes the variability in measurements along the length of the gradients (m). The red dots describe Depth 1 data while the blue diamonds describe the Depth 2 data; the dotted lines describe the % topographical slope.

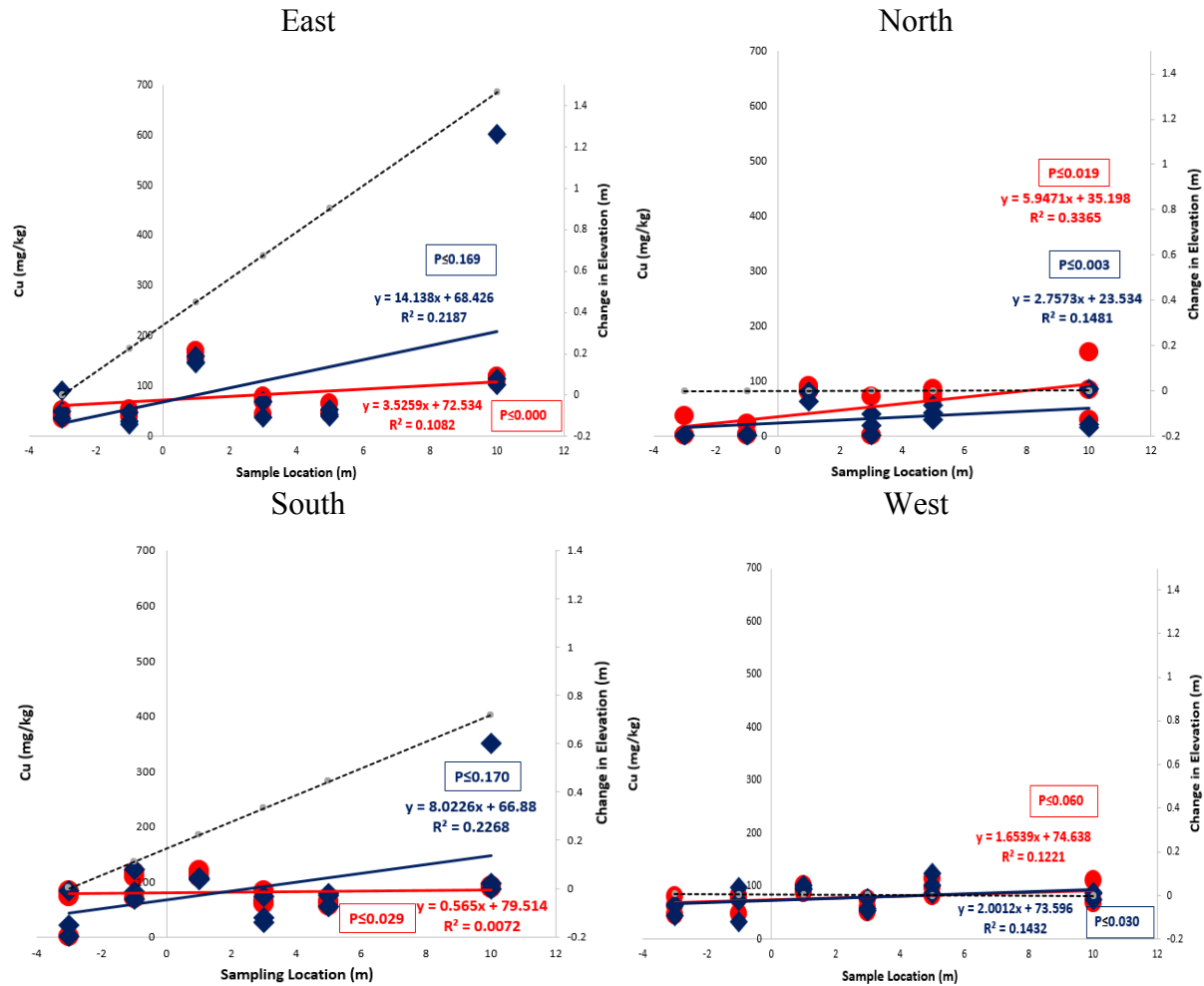


Figure 4.9: Concentration of copper (Cu) in soils across the sampled gradients for both depths at all sampling sites

Variation of Cu measurements for Depth 1 (0-10cm) and Depth 2 (10-20cm) with respect to management intensity/sample location (-3m and -1m at *NatField* and 1m, 3m, 5m and 10m in the *AgField*) and slope with the significant difference (ANOVA, General Linear Model,  $P \leq 0.05$ ).  $R^2$  describes the variability in measurements along the length of the gradients (m). The red dots describe Depth 1 data while the blue diamonds describe the Depth 2 data; the dotted lines describe the % topographical slope.

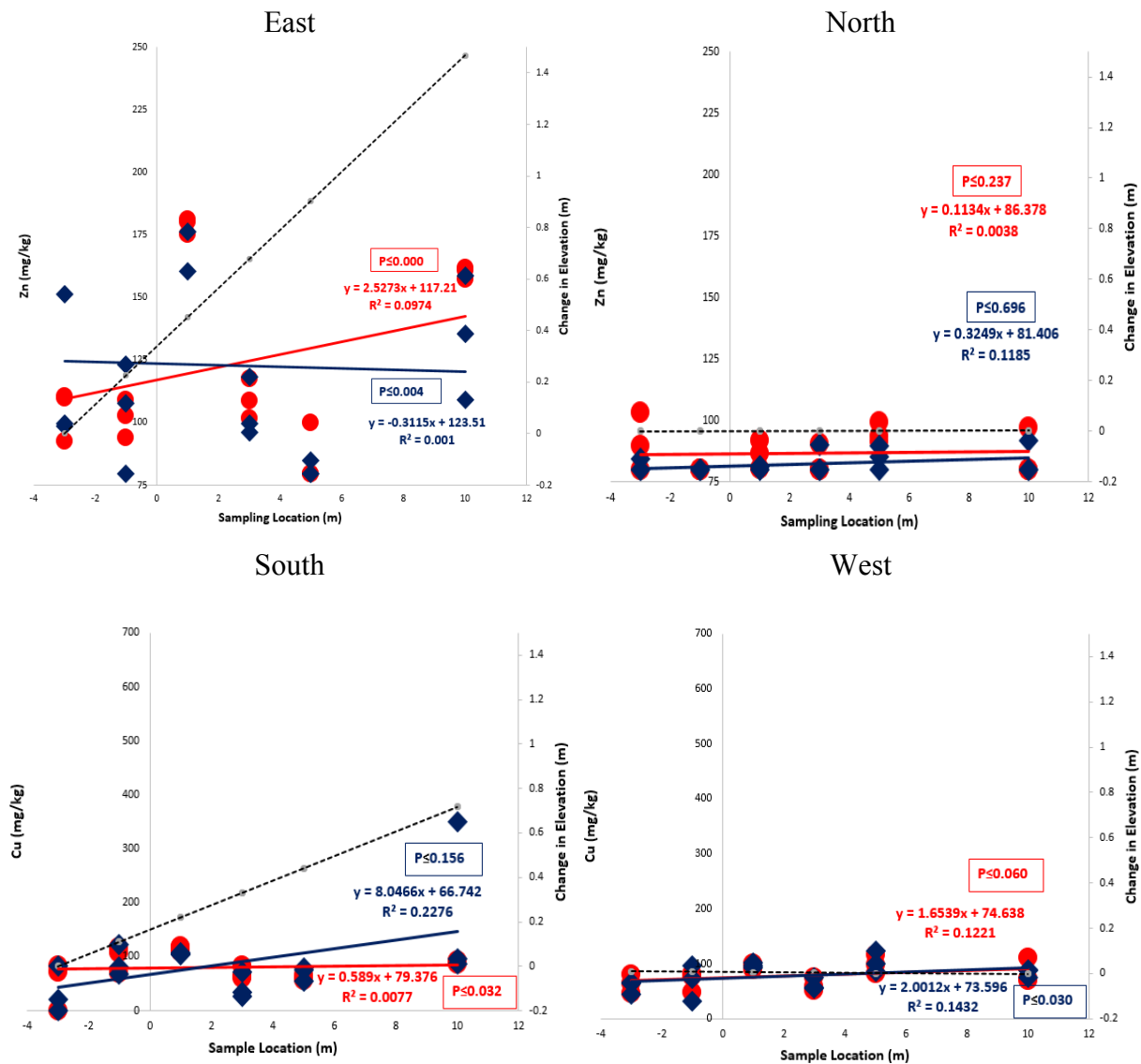


Figure 4.10: Concentration of zinc (Zn) in soils across the sampled gradients for both depths at all sampling sites

Variation of Zn measurements for Depth 1 (0-10cm) and Depth 2 (10-20cm) with respect to management intensity/sample location (-3m and -1m at *NatField* and 1m, 3m, 5m and 10m in the *AgField*) and slope with the significant difference (ANOVA, General Linear Model,  $P \leq 0.05$ ).  $R^2$  describes the variability in measurements along the length of the gradients (m). The red dots describe Depth 1 data while the blue diamonds describe the Depth 2 data; the dotted lines describe the % topographical slope.

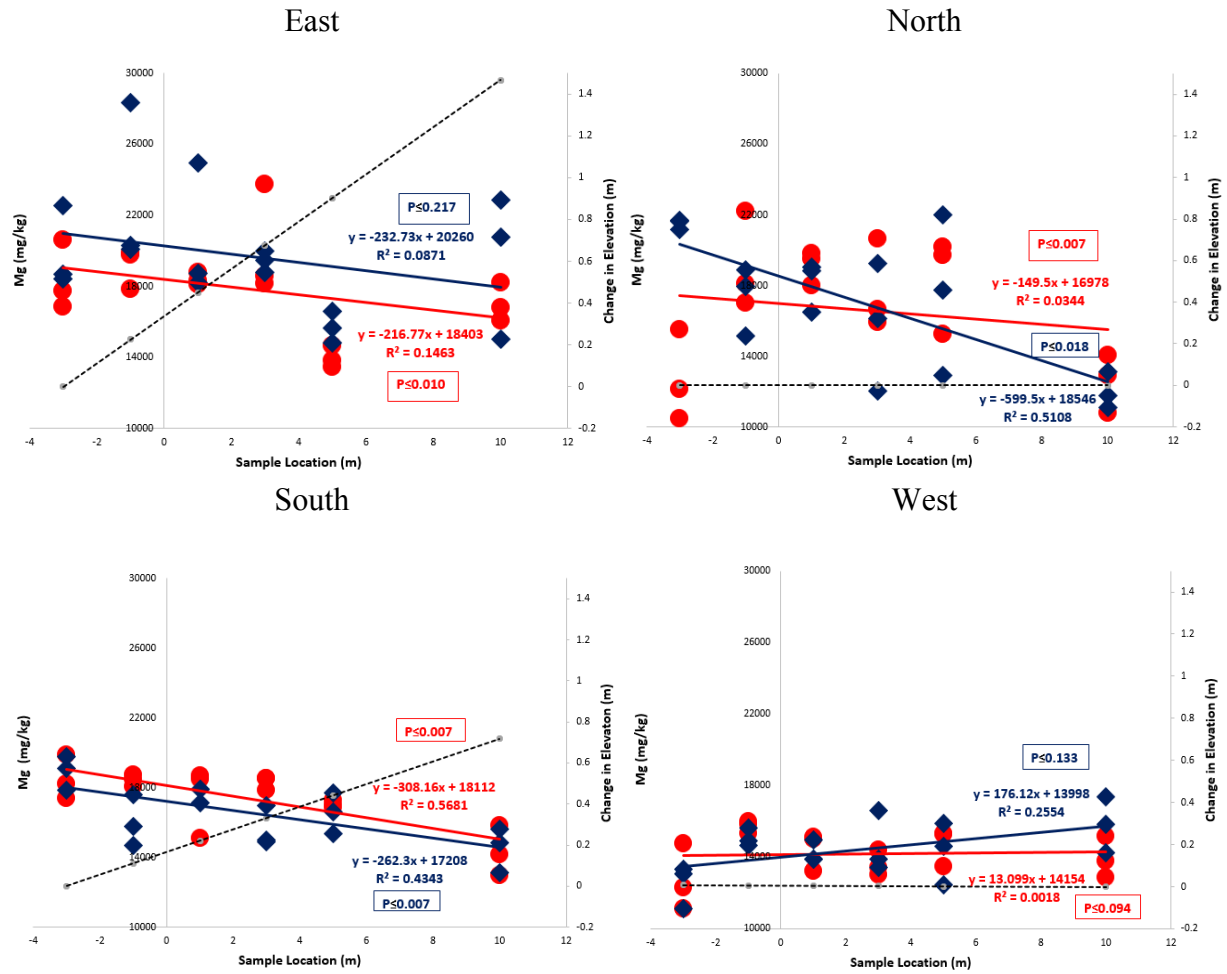


Figure 4.11: Concentration of magnesium (Mg) in soils across the sampled gradients for both depths at all sampling sites

Variation of Mg measurements for Depth 1 (0-10cm) and Depth 2 (10-20cm) with respect to management intensity/sample location (-3m and -1m at *NatField* and 1m, 3m, 5m and 10m in the *AgField*) and slope with the significant difference (ANOVA, General Linear Model,  $P \leq 0.05$ ).  $R^2$  describes the variability in measurements along the length of the gradients (m). The red dots describe Depth 1 data while the blue diamonds describe the Depth 2 data; the dotted lines describe the % topographical slope.



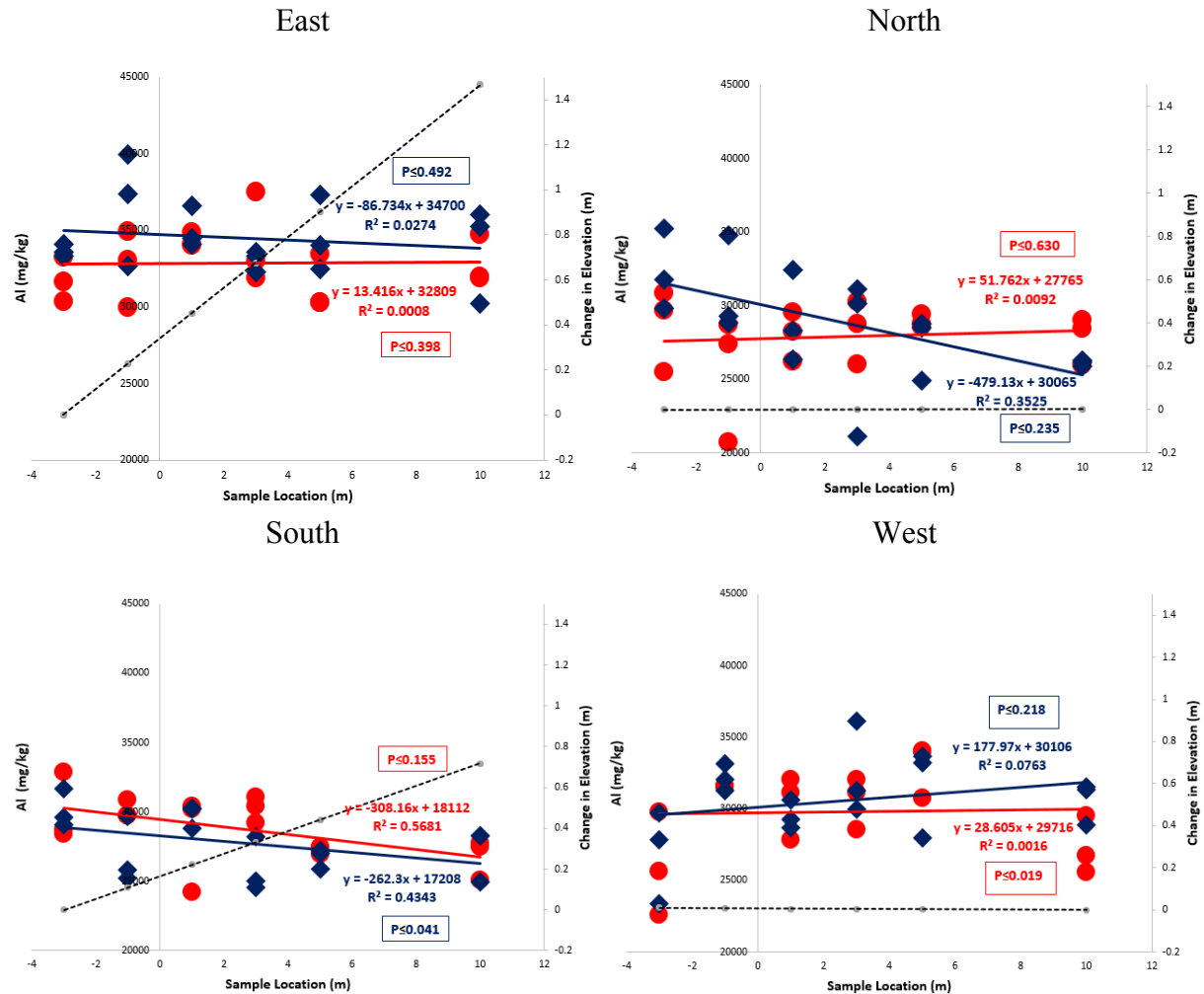


Figure 4.12: Concentration of aluminum (Al) in soils across the sampled gradients for both depths at all sampling sites

Variation of Al measurements for Depth 1 (0-10cm) and Depth 2 (10-20cm) with respect to management intensity/sample location (-3m and -1m at *NatField* and 1m, 3m, 5m and 10m in the *AgField*) and slope with the significant difference (ANOVA, General Linear Model,  $P \leq 0.05$ ).  $R^2$  describes the variability in measurements along the length of the gradients (m). The red dots describe Depth 1 data while the blue diamonds describe the Depth 2 data; the dotted lines describe the % topographical slope.

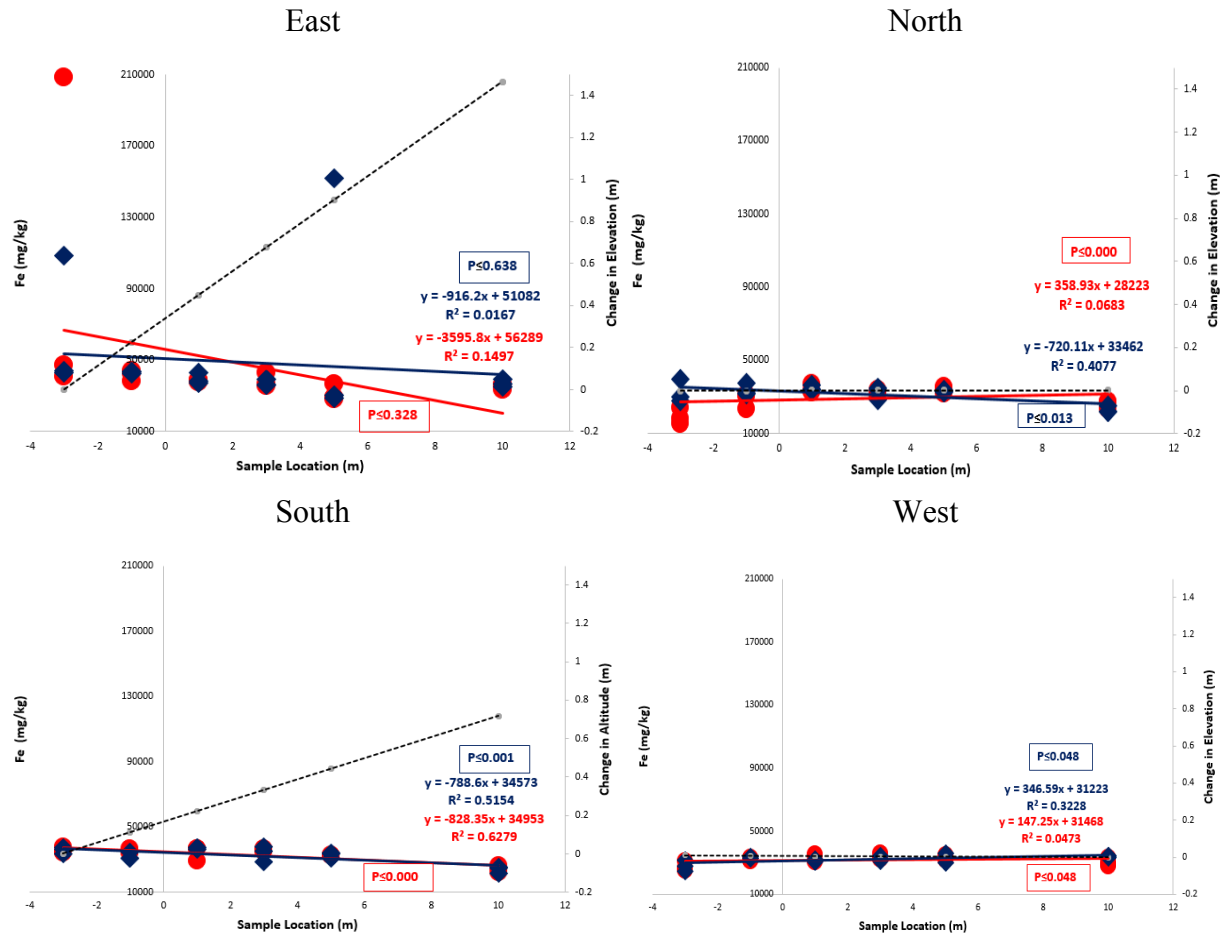


Figure 4.13: Concentration of iron (Fe) in soils across the sampled gradients for both depths at all sampling sites

Variation of Fe measurements for Depth 1 (0-10cm) and Depth 2 (10-20cm) with respect to management intensity/Sample Location (-3m and -1m at *NatField* and 1m, 3m, 5m and 10m in the *AgField*) and slope with the significant difference (ANOVA, General Linear Model,  $P \leq 0.05$ ).  $R^2$  describes the variability in measurements along the length of the gradients (m). The red dots describe Depth 1 data while the blue diamonds describe the Depth 2 data; the dotted lines describe the % topographical slope.

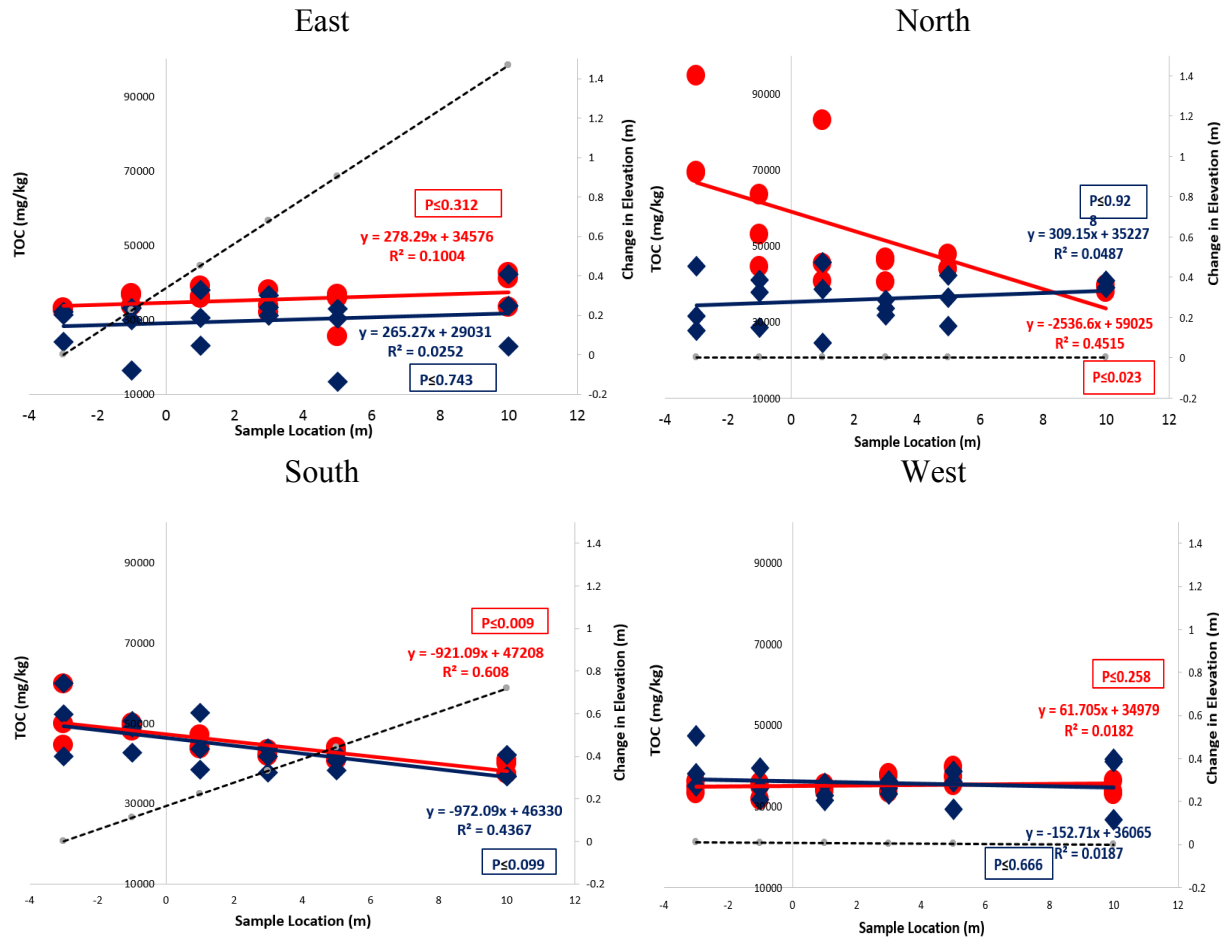


Figure 4.14: Total organic carbon (TOC) in soils across the sampled gradients for both depths at all sampling sites

Variation of TOC measurements for Depth 1 (0-10cm) and Depth 2 (10-20cm) with respect to management intensity/Sample Location (-3m and -1m at *NatField* and 1m, 3m, 5m and 10m in the *AgField*) and slope with the significant difference (ANOVA, General Linear Model,  $P \leq 0.05$ ).  $R^2$  describes the variability in measurements along the length of the gradients (m). The red dots describe Depth 1 data while the blue diamonds describe the Depth 2 data; the dotted lines describe the % topographical slope.

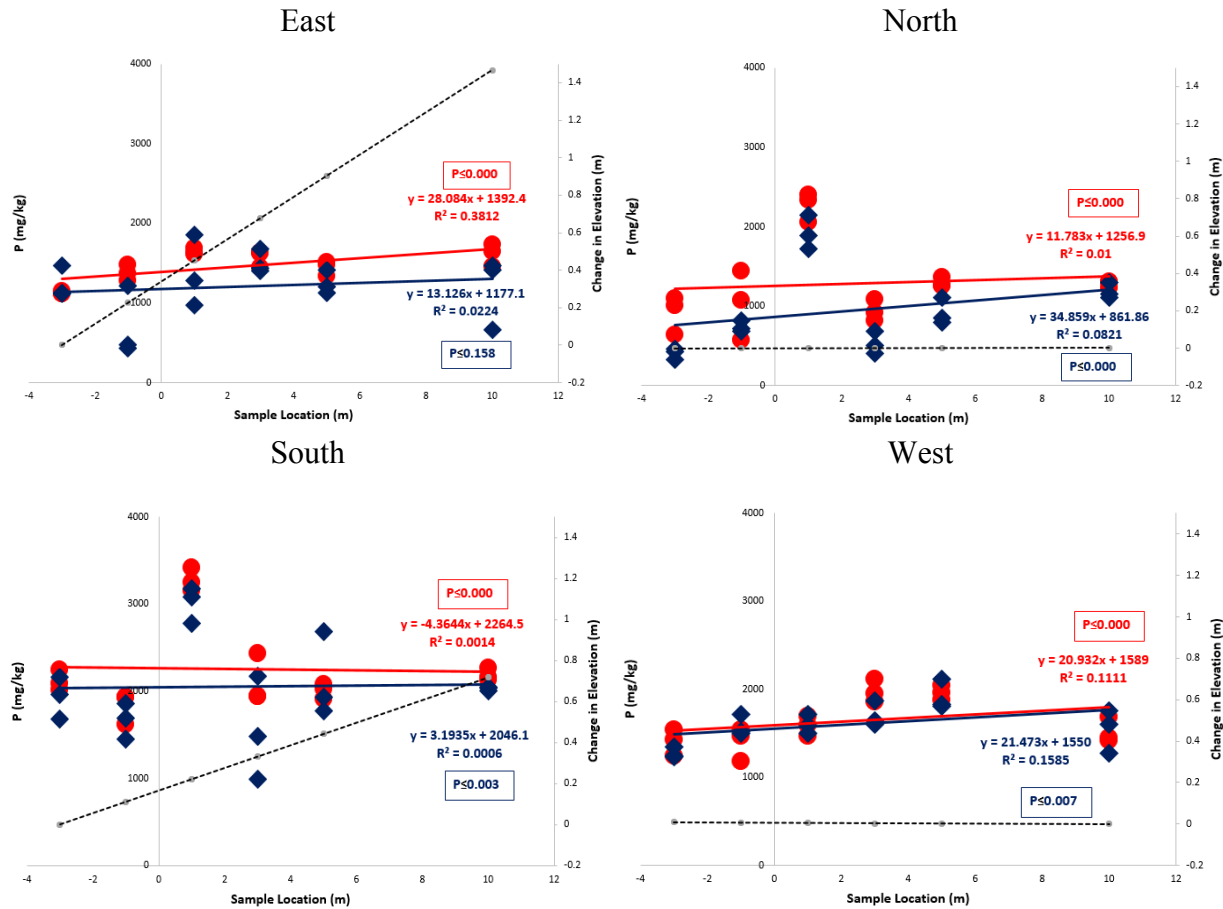


Figure 4.15: Total phosphorus (TP) in soils across the sampled gradients for both depths at all sampling sites

Variation of TP measurements for Depth 1 (0-10cm) and Depth 2 (10-20cm) with respect to management intensity/Sample Location (-3m and -1m at *NatField* and 1m, 3m, 5m and 10m in the *AgField*) and slope with the significant difference (ANOVA, General Linear Model,  $P \leq 0.05$ ).  $R^2$  describes the variability in measurements along the length of the gradients (m). The red dots describe Depth 1 data while the blue diamonds describe the Depth 2 data; the dotted lines describe the % topographical slope.

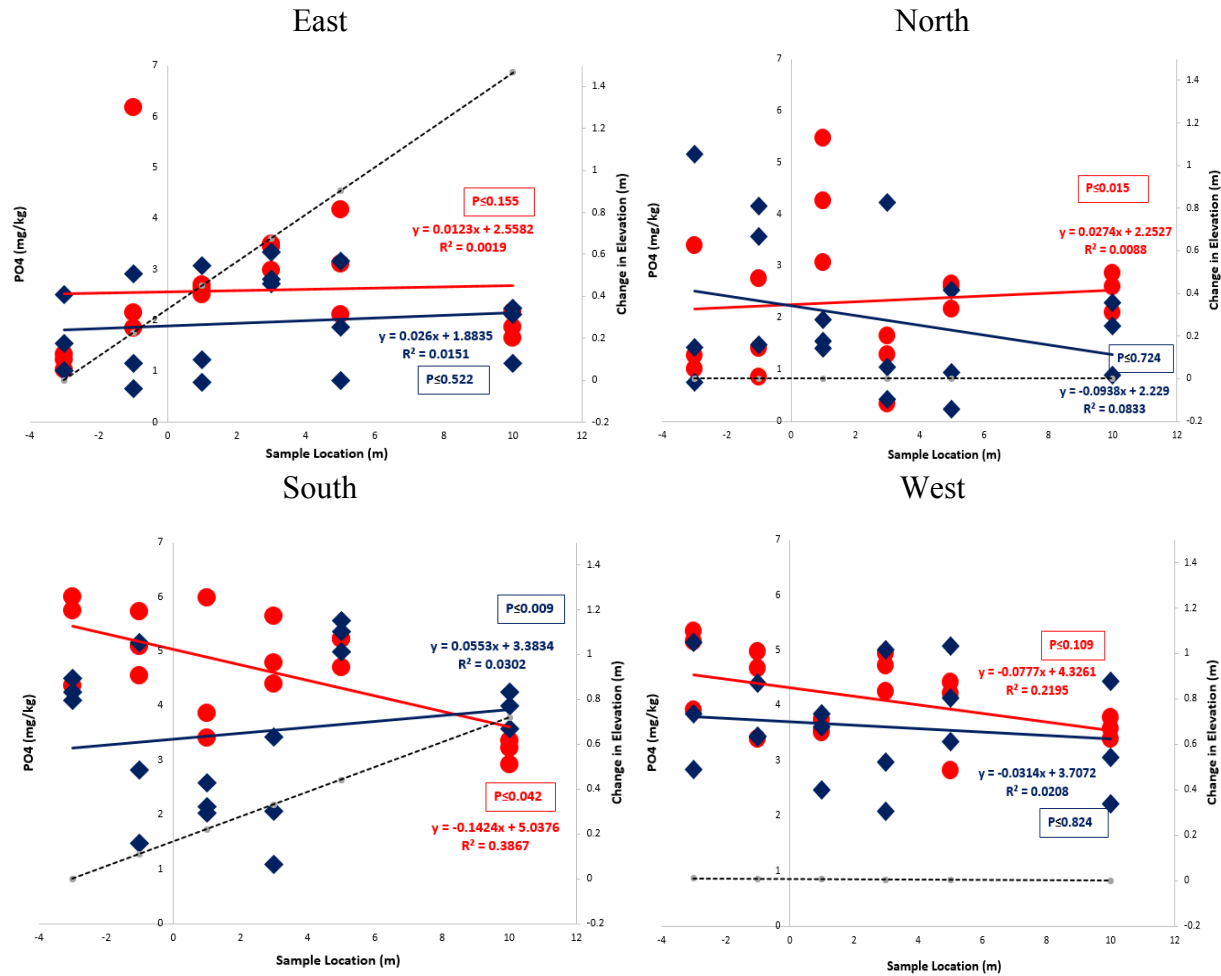


Figure 4.16: Available phosphorus (PO<sub>4</sub>-P) in soils across the sampled gradients for both depths at all sampling sites

Variation of PO<sub>4</sub>-P measurements for Depth 1 (0-10cm) and Depth 2 (10-20cm) with respect to management intensity/Sample Location (-3m and -1m at *NatField* and 1m, 3m, 5m and 10m in the *AgField*) and slope with the significant difference (ANOVA, General Linear Model,  $P \leq 0.05$ ). R<sup>2</sup> describes the variability in measurements along the length of the gradients (m). The red dots describe Depth 1 data while the blue diamonds describe the Depth 2 data; the dotted lines describe the % topographical slope.

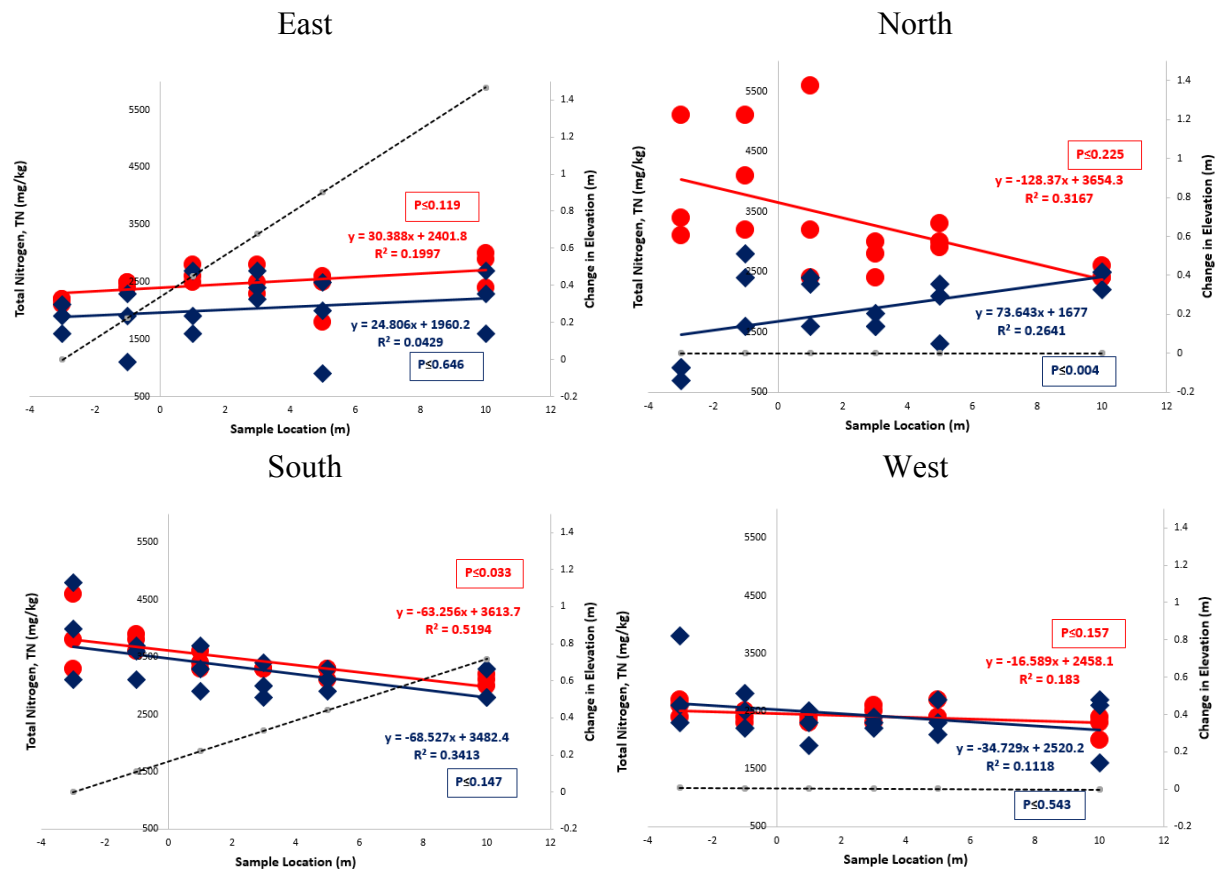


Figure 4.17: Total nitrogen (TN) in soils across the sampled gradients for both depths at all sampling sites

Variation of TN measurements for Depth 1 (0-10cm) and Depth 2 (10-20cm) with respect to management intensity/Sample Location (-3m and -1m at *NatField* and 1m, 3m, 5m and 10m in the *AgField*) and slope with the significant difference (ANOVA, General Linear Model,  $P \leq 0.05$ ).  $R^2$  describes the variability in measurements along the length of the gradients (m). The red dots describe Depth 1 data while the blue diamonds describe the Depth 2 data; the dotted lines describe the % topographical slope.

## 4.2 Similarity of the abiotic properties profiles of the sampled sites and gradient locations

A PCA analysis, carried out in PAST3, that included all the measured physical and chemical parameters listed above, and also including textural parameters allowed for a visualisation of similarity profiles between the test sites (Figure 4.18, a and Figure 4.19, a) and gradient locations (Figure 4.18, b and Figure 4.19, b)

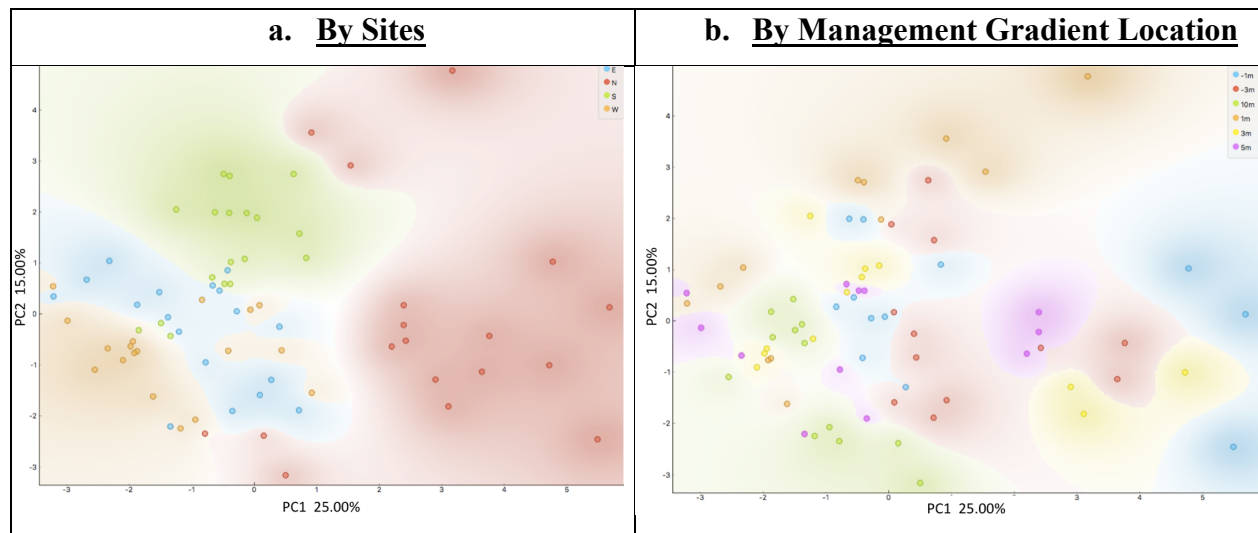


Figure 4.18: Similarity of soil samples' chemical-physical and textural profiles as described by Principal Component Analysis (PCA). Data for Depth 1.

Figure 4.18 describes similarity profile across a) sampled site (i.e. East, West, North, and South). and b) sampled management gradient location (i.e. *NatField* to *AgField*: -3m, -1m, 1m, 3m, 5m, 10m). While each site is distinct from the others this is most obvious for the North site. On the other hand, the dissimilarity along the management gradient locations while less immediately evident it does suggest that the inner most *AgField* location, -10m (bottom left, green) is distinct from the other gradient locations.

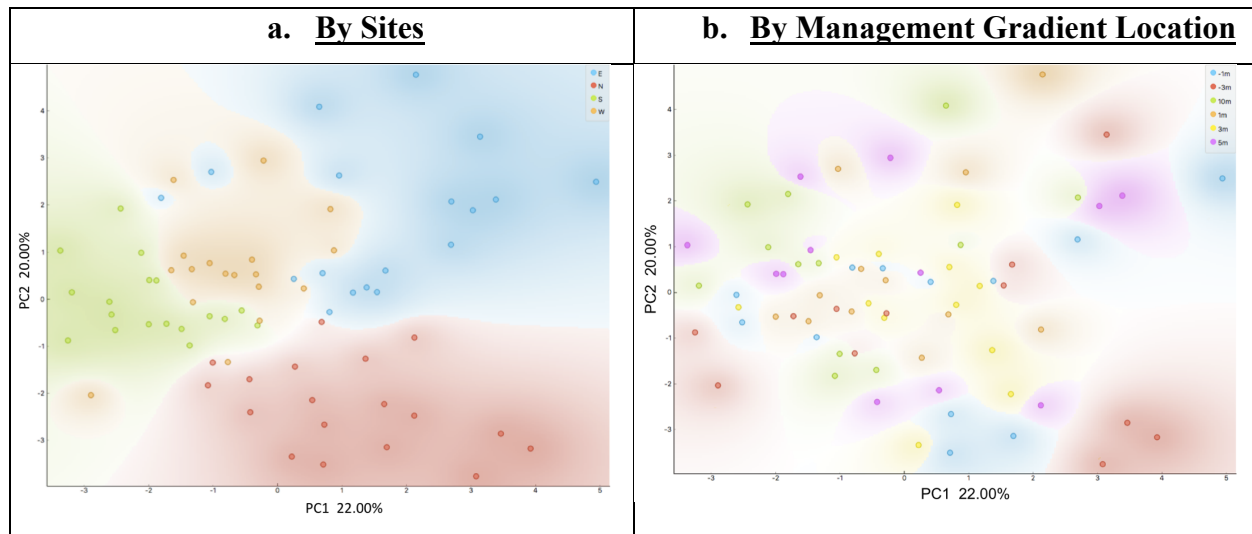


Figure 4.19: Similarity of soil samples' chemical profiles across sampling sites as described by Principal Component Analysis (PCA). Data for Depth 2

Top image describes similarity profile across sampled sites (i.e. East, West, North, and South).  
 Bottom image describes similarity profiles along the sampling location (i.e. *NatField* to *AgField*: -3m, -1m, 1m, 3m, 5m, 10m)

The Depth 1 pattern is mainly due to variability in pH and primary nutrients such as TN, P and K, and TOC, whereas for Depth 2, the pattern is driven by clay (%), cations such as Al, Mg and Fe, and micronutrients such as Cu and Zn (see Appendix 7.17).



### 4.3 Alpha Diversity

Alpha diversity, in ecological terminology, describes the richness and abundance of a population within a defined environment. For this experiment, alpha diversity parameters have been estimated within sites and within locations along the management gradient.

#### *Bacterial alpha diversity*

Bacterial alpha diversity assessments were carried out at OTU level. In general, it is difficult to glean any notable differences in the alpha diversity parameters across the sampled sites and gradients (see [Figure 4.20](#)).

For Depth 1, the Dominance index ranges from 0.004 for South site *NatField* (-3m) to 0.009 for the North site *AgField* (5m) whereas at Depth 2, the index ranges from 0.004 for South site *NatField* (-1m) to 0.009 in the West site *AgField* (10m).

For Depth 1, the Simpson index ranges from 0.991 in North site of *AgField* (5m) to 0.996 in the South site *NatField* (-3m) whereas at Depth 2, the index ranges from 0.991 for West site *AgField* (10m) to 0.996 for the South site *NatField* (-1m).

For Depth 1, Shannon index ranges from 4.710 in North site *AgField* (5m) to 5.587 in the South site of *NatField* (-3m) whereas for Depth 2, the index ranges from 4.710 for West site *AgField* (10m) to 5.489 for the South site *NatField* (-1m).

For Depth 1, Chao index ranges from 6216 in North site of *AgField* (5m) to 35780 in the South site of *NatField* (-3m) whereas at Depth 2, Chao index ranges from in 6216 West site of *AgField* (10m) to 29400 in the South site of *NatField* (-1m).

Form  
Delet

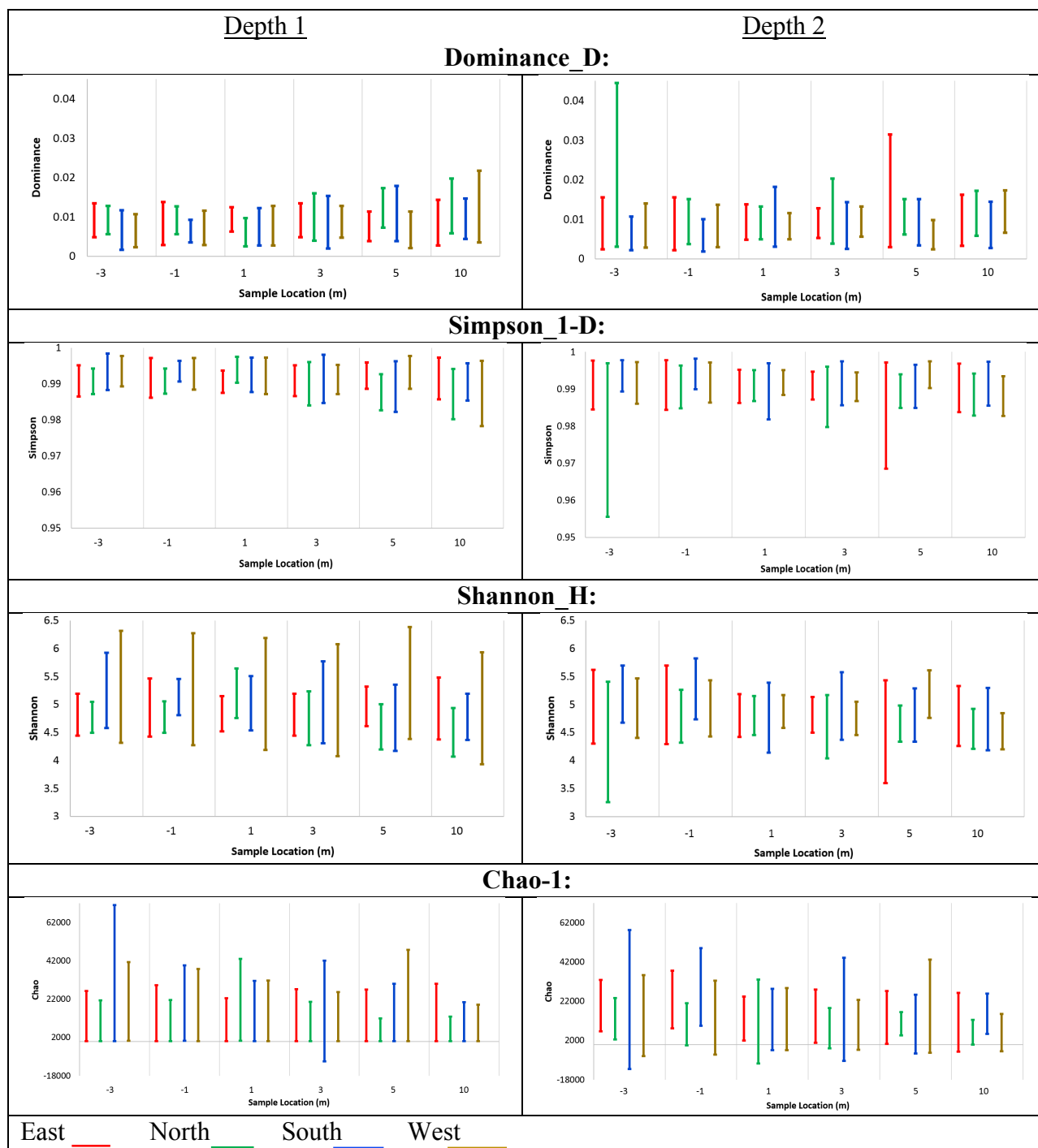


Figure 4.20: Alpha diversity indices for the bacterial community. Indices calculated using OTU abundance data.

Each bar describes the distance between the upper maximum and lower minimum values for each condition.

---

### *Fungal alpha diversity*

Fungal alpha diversity assessments were carried out at OTU level. In general, it is difficult to glean any notable differences in the alpha diversity parameters across the sampled sites and gradients (see [Figure 4.21](#)).

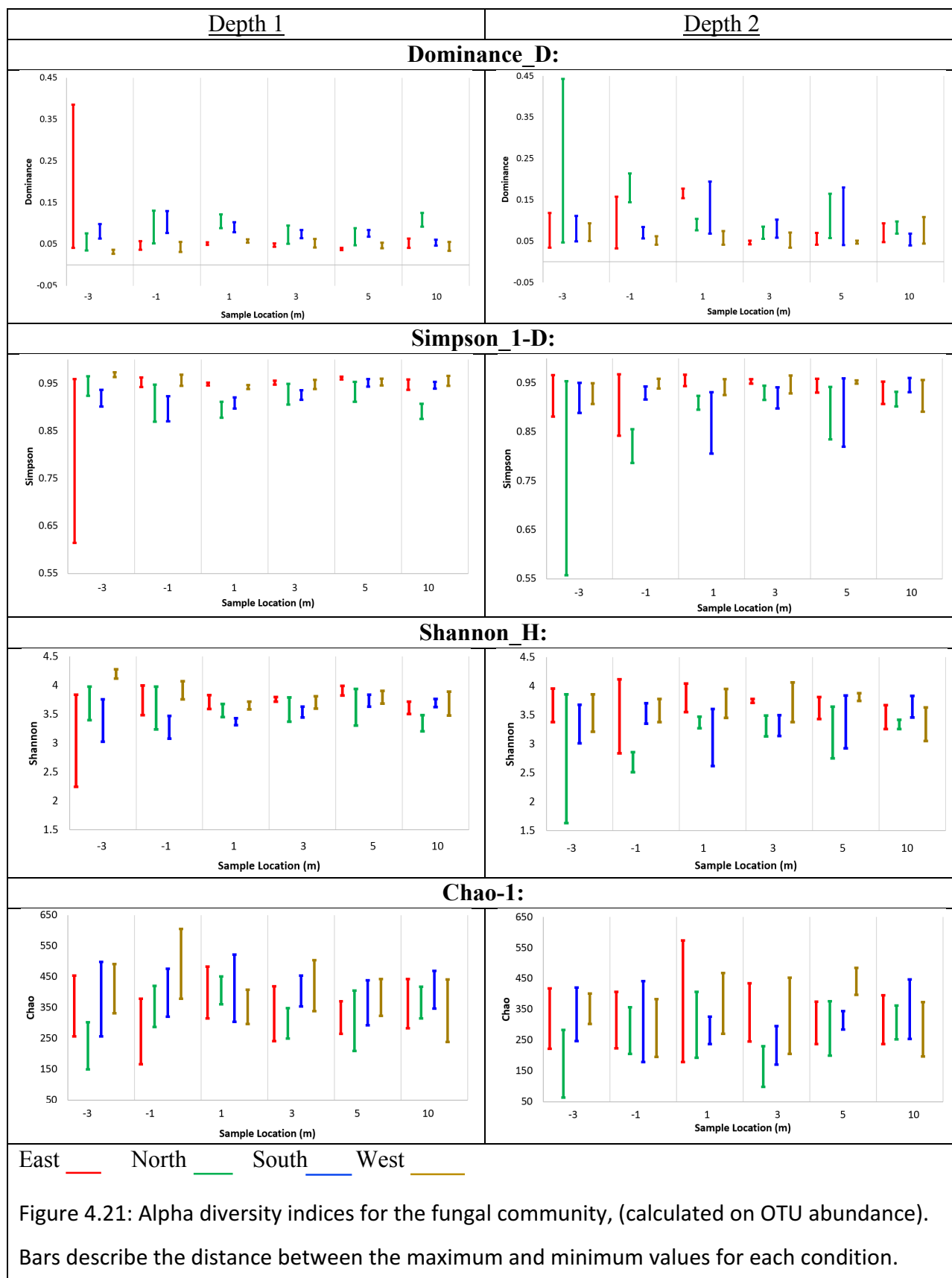
For Depth 1, Dominance index ranges from 0.028 in the West of *NatField* (-3m) to 0.128 in North site of *NatField* (-1m) whereas at Depth 2, index ranges from 0.035 in West site of *AgField*(3m) to 0.192 in the South site of *AgField*(1m).

For Depth 1, Simpson index ranges from 0.872 in North site of *NatField* (-3m) to 0.973 in the West site of *NatField* (-1m) whereas at Depth 2, index ranges from 0.808 in South site of *AgField* (1m) to 0.967 in the East site of *AgField*(1m).

For Depth 1, Shannon index ranges from 3.092 in South site of *NatField* (-1m) to 4.258 in the West site of *NatField* (-3m) whereas at Depth 2, index ranges from 2.634 in South site of *AgField*(1m) to 4.039 in the West site of *AgField*(3m).

For Depth 1, Chao index ranges from 172 in North site of *NatField* (-3m) to 508.2 in the South site of *AgField*(1m) whereas at Depth 2, index ranges from in 200.1 South site of *NatField* (-1m) to 510.1 in the East site of *AgField*(1m).

Form  
Delet



#### 4.4 Beta Diversity

Beta diversity is an ecological term used to describe differences between populations. Here beta diversity was used to estimate the similarities and dissimilarities between sites and sample locations along the management gradients. Beta diversity analyses were carried out using multiple approaches as detailed in the Methodology section.

##### *Bacterial beta diversity*

An initial analysis employed a Bray-Curtis similarity index approach; to assess the changes in diversity as compared to the least managed location within the *NatField* natural field the sampling location -3m was to all other sampling locations (-1m, 1m, 3m, 5m and 10m). An internal diversity comparison, i.e. among the -3m samples, was carried out to offer a comparative baseline to any shift in diversity larger than the internal diversity structure (see [Figure 4.22](#), and [Error! Reference source not found.](#)). The similarity in bacterial diversity to -3m decreased towards the inner field locations from *NatField* to *AgField* for all gradients and both depths, with the exception of except at Depth 1 of East and North Site. However, this might be due to a larger than expected variability within the -3m datasets at the two sites; barring this the trend was generally also decreasing along the gradient as described from the -1m to the 10m location. A SIMPER analysis, was carried out employing the taxonomic class level datasets. This analysis allowed an estimate of the proportional role of each class in determining dissimilarities among locations along the *NatField* to *AgField* gradient. This also allowed for a calculation of the rate of change in the proportional abundance of each taxon expressed in proportional abundance per distance (m) away from the -3m location. The classes that were identified as being the top five drivers of dissimilarity among gradient locations are shortly discussed. It should be noted that the proportional abundance for any one class was relatively small (See Appendix 8.1).

At -3m, the mostly found taxa in the largest proportional abundance for all sites are *Flavobacteriia* class, *Ignavibacteria* class, *Gemmatimonadetes* phylum and *Betaproteobacteria* class. At 10m, the mostly found taxa in the largest proportional abundance for all sites are *Deinococcus* class, *Acidobacteriia* class, *Thermomicrobia* phylum, *Thermoleophilia* class, *Epsilonproteobacteria* class, *Holophagae* class, *Ktedonobacteria* class, *Synergistia* class, *Actinobacteria* phylum and *Bacteroidia* class (See Appendix 8.1).

For Depth 1 *Deinococci* class were found to be in the largest proportional abundance for all sites except West while for Depth 2, the same was true for the South and West sites.

Table 4.1: Changes in the level of similarity of bacterial communities along the sampled *NatField* to *AgField* gradients as described by the change in the Bray-Curtis similarity index value along the sampled gradient versus the -3m location

Sites	R <sup>2</sup> at Depth 1	Equation for Depth 1	R <sup>2</sup> at Depth 2	Equation for Depth 2
East	2.6 (+)	$y = 0.0028x + 0.3319$	11.64 (-)	$y = -0.0026x + 0.5676$
North	37.25 (+)	$y = 0.0053x + 0.279$	8.06 (-)	$y = -0.0021x + 0.396$
South	87.12 (-)	$y = -0.0085x + 0.633$	17.02 (-)	$y = -0.0088x + 0.552$
West	96.75 (-)	$y = -0.015x + 0.6246$	63.68 (-)	$y = -0.0061x + 0.5632$
Similarity increasing (+)				
Similarity decreasing (-)				
R <sup>2</sup> represents the strength of the correlation between changes in the Bray-Curtis similarity index with the distance from the -3m location.				

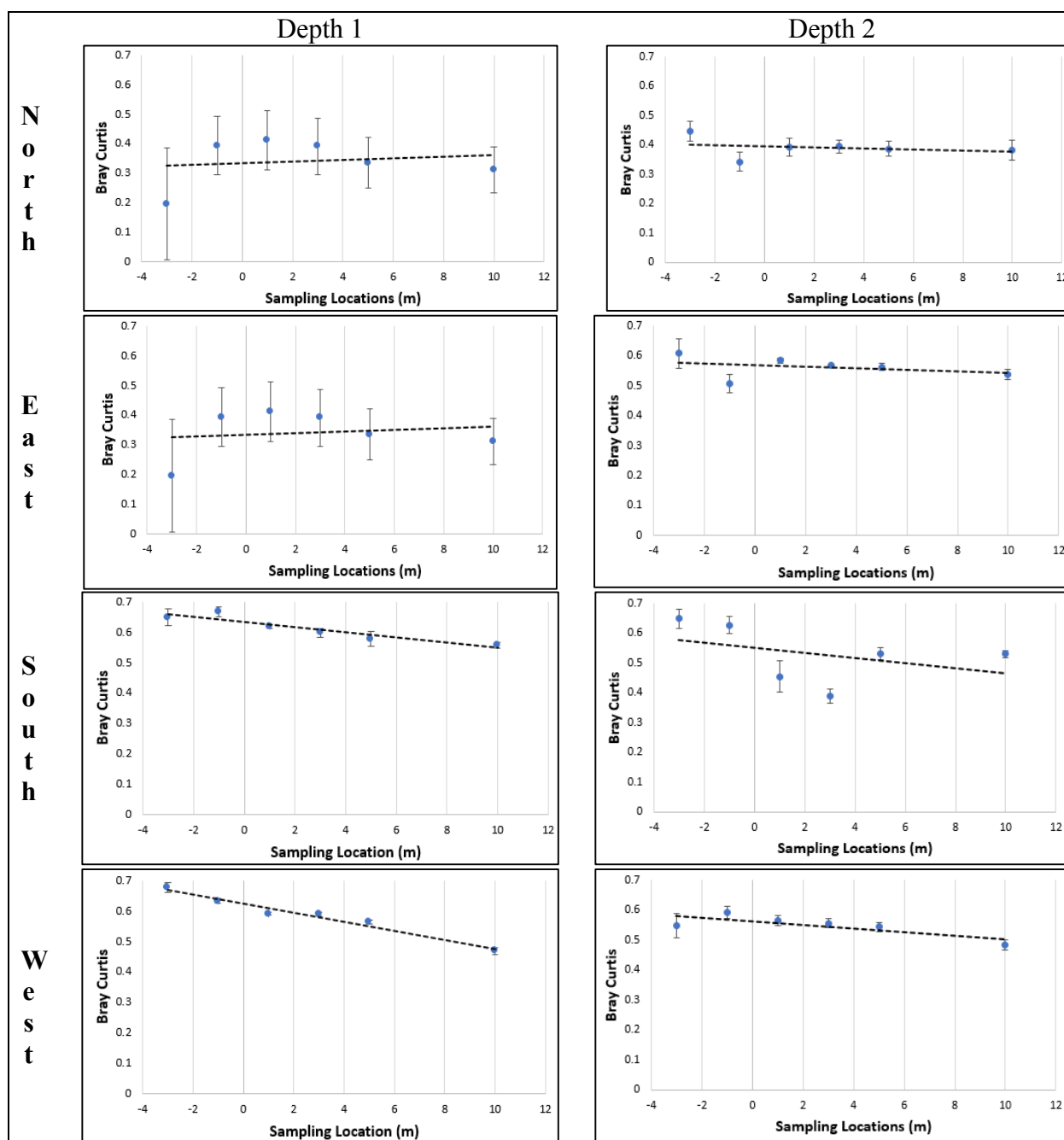


Figure 4.22: Comparison of microbial diversity along the *NatField* to *AgField* gradient; change in the Bray-Curtis similarity index value along the sampled gradient versus the -3m location. Error bars describe the 95% CI.

### *Fungal beta diversity*

Fungal Beta Diversity was carried out at OTU level. Here, sampling location -3m (natural field) was compared with itself and other sampling locations (-3, -1m, 1m, 3m, 5m and 10m). Each point on the above graph is the mean of transects (A, B and C) of each site (North, South, East and West) at each sampling locations. Here the diversity of bacteria was decreasing from *NatField* to *AgField* in the North, South and West site at both the depths whereas in the East, the Beta diversity was increasing at Depth 1 and no change at Depth 2 (see [Figure 4.21](#), and [Table 4.2](#)).

Rate of change in the proportional abundance of bacterial taxa was done at class level. The Taxa was identified as top five drivers of dissimilarity among sites, via SIMPER analysis (Past3) (Hammer, 2017).

At -3m, taxa in the largest proportional abundance for all sites are *Saccharomycetes* class, *Lecanoromycetes* class, *Agaricomycetes* class and *Dacrymycetes* class. At 10m, taxa in the largest proportional abundance for all sites are *Ustilaginomycetes* class, *Wallemiomycetes* class, *Pezizomycetes* class, *Chytridiomycetes* class, *Tremellomycetes* class, *Agaricostilbomycetes* class, *Monoblepharidomycetes* class, *Leotiomyces* class and *Cystobasidiomycetes* class (See Appendix 8.2)

Form

Delete

Form

Delete



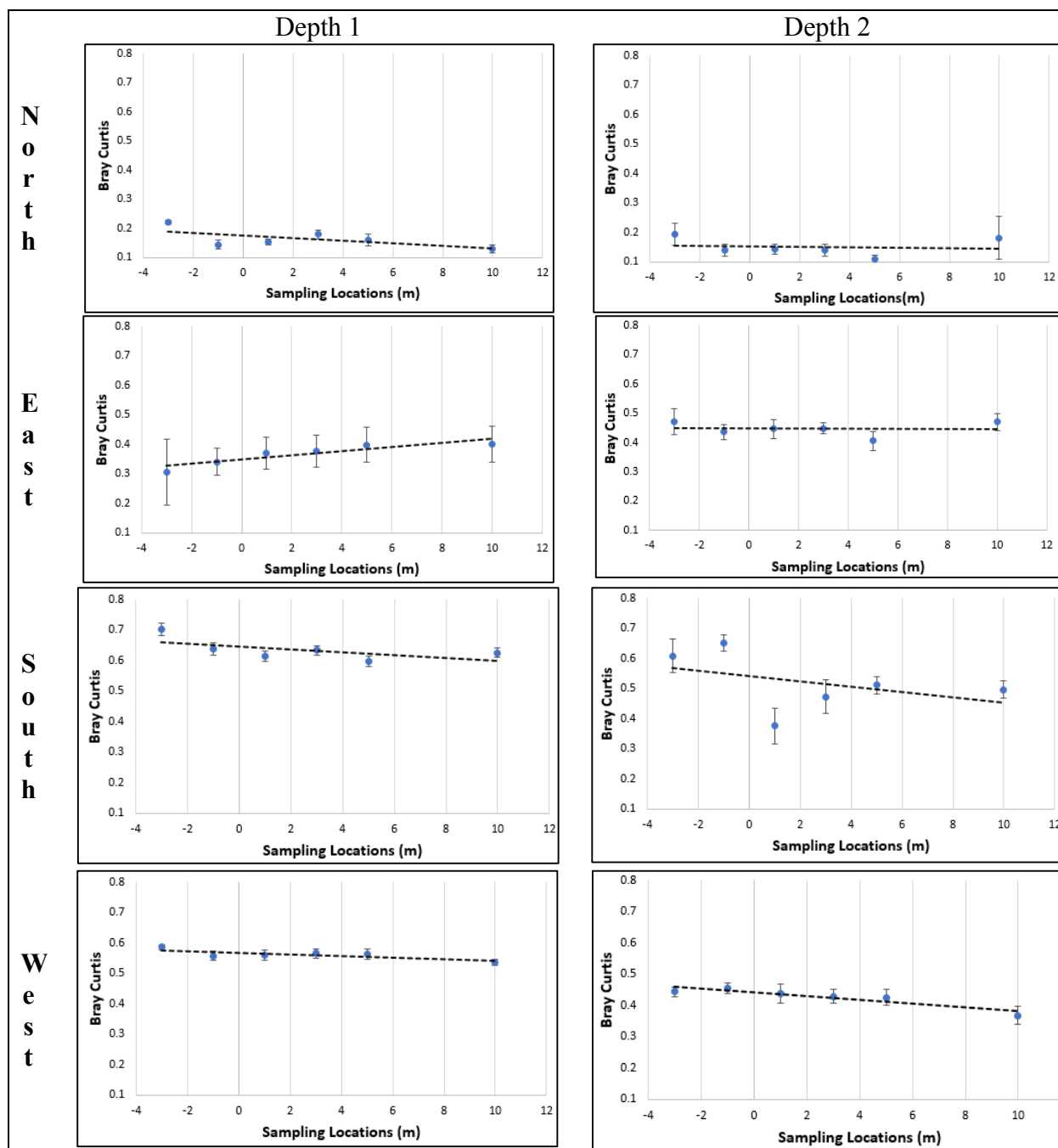


Figure 4.23: Comparison of microbial diversity along the *NatField* to *AgField*; change in the Bray-Curtis similarity index value along the sampling location versus the -3m sampling location. Error bars describe the 95% CI.

Table 4.2: Changes in the level of similarity between fungal communities along the sampled *NatField* to *AgField*; change in the Bray-Curtis similarity index value along the sampled gradient versus the -3m location

Sites	R <sup>2</sup> at Depth 1	Equation for Depth 1	R <sup>2</sup> at Depth 2	Equation for Depth 2
East	80.56 (+)	$y = 0.007x + 0.3479$	0 (-)	$y = -0.0003x + 0.4472$
North	43.08 (-)	$y = -0.0046x + 0.1761$	1.06 (-)	$y = -0.0007x + 0.1532$
South	37.99 (-)	$y = -0.0049x + 0.6469$	16.93 (-)	$y = -0.0088x + 0.5413$
West	66.05 (-)	$y = -0.0028x + 0.5685$	84.23 (-)	$y = -0.0061x + 0.4416$
Similarity increasing (+) Similarity decreasing (-) R <sup>2</sup> represents the strength of the correlation between changes in the Bray-Curtis similarity index with the distance from the -3m location.				

#### 4.4.1 Predictive discriminant analyses

A non-traditional way to assess beta diversity indirectly is to employ brute force self-learning and multiple regression approaches to determine the similarity level among tested conditions. These approaches allow for the estimation of likely similarities among complex populations. Neural network and logistic regression tools were employed as described in the Methodology.

All such similarities or dissimilarities, and thus prediction success rates, are expected to be a function of the conditions induced by site and location along the management gradient.

These prediction exercises have shown that in general the gradient locations are most similar to locations in their immediate neighborhood. For example, while employing the abiotic parameters (Figure 4.24) a Neural Network (NN) confusion matrix for depth 1, shows that only 4 out of 12 samples collected at -3 m were correctly identified. However, another 7 out of 12 were assigned to the immediately adjoining location, at -1 m. Thus 11 out of 12 samples were assigned to the general -3 m to -1 m region of the sampled gradient. One must note that this analysis bins all samples collected at one gradient location for all 4 sites. However, for the true -1 m samples only 50% (6 out of 6) were identified in the -3 m to -1 m region. As these samples are near the *NatField/ AgField* boundary this suggests similarities across the boundary. On the other hand, far inside the *AgField* 11 out of 12 samples are correctly assigned, an indication of the unique abiotic environmental parameters. Again, given the fact that these samples represent all four sites (N, E, S and W) this is an indication of the role of management in smoothing differences in soil's parameters.

It should be noted that the two predictive algorithms, the NN and Logistic regression (LR) were best suited to predict location of samples along the management gradient. Therefore, both are listed here, for all discriminant analyses.

A prediction exercise has shown that in general the gradient locations are most similar to locations in their immediate neighborhood. Analysis carried out on the bacterial taxonomic diversity has confirmed that the similarity between sites extends strongly to the immediate vicinity, with commonly 10 to 11 out of 12 samples being correctly assigned or assigned to the neighboring sampling locations along the gradient. Moreover, the discriminant analysis identified with accuracy close to 100%, nearly always, the 10m samples. This confirms that there

Form  
Delet

is continuity along the gradient and that the inner-field sites do have a distinct bacterial population structure ([Figure 4.25](#)).

The same was true for fungal-based analysis, but the trend was only clearly noticeable for the LR analysis versus the NN tests ([Figure 4.26](#)).

The same was true for functional diversity structure as that of bacterial taxonomic diversity, which confirms that there is continuity along the gradient and that the inner-field sites do have a distinct functional diversity structure ([Figure 4.27](#)).

Delet  
Form

Delet  
Form

Form  
Delet

<u>Depth 1 (Neural Network)</u>														
Predicted														
		1 (-3m)	2 (-1m)	3 (1m)	4 (3m)	5 (5m)	6 (10m)	Σ	Gradient location	AUC	CA	F1	Precision	Recall
Actual	1 (-3m)	4	7	0	0	1	0	12	-3 m	0.690	0.819	0.381	0.444	0.333
	2 (-1m)	4	4	1	2	1	0	12	-1 m	0.660	0.750	0.308	0.286	0.333
	3 (1m)	0	0	11	0	0	1	12	1 m	0.980	0.972	0.917	0.917	0.917
	4 (3m)	0	1	0	6	5	0	12	3 m	0.810	0.833	0.500	0.500	0.500
	5 (5m)	1	2	0	3	6	0	12	5 m	0.795	0.819	0.480	0.462	0.500
	6 (10m)	0	0	0	1	0	11	12	10 m	1.000	0.972	0.917	0.917	0.917
	Σ	9	14	12	12	13	12	72						

<u>Depth 1 (Logistic Regression)</u>														
Predicted														
		1 (-3m)	2 (-1m)	3 (1m)	4 (3m)	5 (5m)	6 (10m)	Σ	Gradient location	AUC	CA	F1	Precision	Recall
Actual	1 (-3m)	8	2	0	0	2	0	12	-3 m	0.885	0.861	0.615	0.571	0.667
	2 (-1m)	4	2	0	3	3	0	12	-1 m	0.485	0.736	0.174	0.286	0.167
	3 (1m)	1	0	8	0	1	2	12	1 m	0.920	0.903	0.696	0.727	0.667
	4 (3m)	1	3	1	4	3	0	12	3 m	0.650	0.736	0.296	0.267	0.333
	5 (5m)	0	4	1	6	1	0	12	5 m	0.585	0.722	0.091	0.100	0.083
	6 (10m)	0	0	1	2	0	9	12	10 m	0.970	0.931	0.783	0.818	0.750
	Σ	14	11	11	15	10	11	72						

<u>Depth 2 (Neural Network)</u>														
Predicted														
		1 (-3m)	2 (-1m)	3 (1m)	4 (3m)	5 (5m)	6 (10m)	Σ	Gradient location	AUC	CA	F1	Precision	Recall
Actual	1 (-3m)	4	5	0	1	2	0	12	-3 m	0.884	0.792	0.348	0.364	0.333
	2 (-1m)	5	3	0	3	1	0	12	-1 m	0.582	0.792	0.286	0.333	0.250
	3 (1m)	0	0	10	0	2	0	12	1 m	0.963	0.972	0.909	1.000	0.833
	4 (3m)	0	1	0	7	4	0	12	3 m	0.873	0.792	0.483	0.412	0.583
	5 (5m)	2	0	0	6	4	0	12	5 m	0.792	0.764	0.320	0.308	0.333
	6 (10m)	0	0	0	0	0	12	12	10 m	1.000	1.000	1.000	1.000	1.000
	Σ	11	9	10	17	13	12	72						

<u>Depth 2 (Logistic Regression)</u>														
Predicted														
		1 (-3m)	2 (-1m)	3 (1m)	4 (3m)	5 (5m)	6 (10m)	Σ	Gradient location	AUC	CA	F1	Precision	Recall
Actual	1 (-3m)	8	1	0	0	3	0	12	-3 m	0.940	0.889	0.667	0.667	0.667
	2 (-1m)	2	2	0	5	3	0	12	-1 m	0.613	0.722	0.167	0.167	0.167
	3 (1m)	1	2	4	0	1	4	12	1 m	0.937	0.847	0.421	0.571	0.333
	4 (3m)	1	4	1	4	2	0	12	3 m	0.588	0.736	0.296	0.267	0.333
	5 (5m)	0	3	0	5	4	0	12	5 m	0.617	0.722	0.286	0.250	0.333
	6 (10m)	0	0	2	1	3	6	12	10 m	0.847	0.861	0.545	0.600	0.500
	Σ	12	12	7	15	16	10	72						

Figure 4.24: Discriminant analysis of gradient locations based on the structure of the **abiotic factors**. Confusion matrices and prediction quality parameters (see Methodology).

<u>Depth 1 (Neural Network)</u>															
		Predicted													
		1 (-3m)	2 (-1m)	3 (1m)	4 (3m)	5 (5m)	6 (10m)	Σ							
Actual	1 (-3m)	6	4	0	0	1	1	12	Gradient location	AUC	CA	F1	Precision	Recall	
	2 (-1m)	0	8	3	1	0	0	12		-3 m	0.830	0.917	0.667	1.000	0.500
	3 (1m)	0	5	6	0	1	0	12		-1 m	0.745	0.792	0.516	0.421	0.667
	4 (3m)	0	0	2	4	4	2	12		1 m	0.780	0.819	0.480	0.462	0.500
	5 (5m)	0	2	2	4	2	2	12		3 m	0.685	0.819	0.381	0.444	0.333
	6 (10m)	0	0	0	0	0	12	12		5 m	0.630	0.778	0.200	0.250	0.167
Σ		6	19	13	9	8	17	72	10 m	1.000	0.931	0.828	0.706	1.000	

<u>Depth 1 (Logistic Regression)</u>															
		Predicted													
		1 (-3m)	2 (-1m)	3 (1m)	4 (3m)	5 (5m)	6 (10m)	Σ							
Actual	1 (-3m)	5	3	2	0	1	1	12	Gradient location	AUC	CA	F1	Precision	Recall	
	2 (-1m)	1	7	3	1	0	0	12		-3 m	0.945	0.889	0.556	0.833	0.417
	3 (1m)	0	3	6	1	2	0	12		-1 m	0.785	0.833	0.538	0.500	0.583
	4 (3m)	0	0	2	4	6	0	12		1 m	0.750	0.792	0.444	0.400	0.500
	5 (5m)	0	1	2	5	4	0	12		3 m	0.780	0.778	0.333	0.333	0.333
	6 (10m)	0	0	0	1	1	10	12		5 m	0.730	0.750	0.308	0.286	0.333
Σ		6	14	15	12	14	11	72	10 m	0.980	0.958	0.870	0.909	0.833	

<u>Depth 2 (Neural Network)</u>															
		Predicted													
		1 (-3m)	2 (-1m)	3 (1m)	4 (3m)	5 (5m)	6 (10m)	Σ							
Actual	1 (-3m)	6	2	3	0	0	1	12	Gradient location	AUC	CA	F1	Precision	Recall	
	2 (-1m)	4	3	2	0	3	0	12		-3 m	0.883	0.831	0.522	0.545	0.500
	3 (1m)	1	4	3	1	3	0	12		-1 m	0.641	0.746	0.308	0.286	0.333
	4 (3m)	0	0	6	4	2	0	12		1 m	0.592	0.690	0.083	0.083	0.083
	5 (5m)	0	1	4	2	5	0	12		3 m	0.696	0.803	0.222	0.333	0.167
	6 (10m)	0	0	0	0	0	11	11		5 m	0.799	0.775	0.200	0.250	0.167
Σ		11	10	18	7	13	12	71	10 m	1.000	0.887	0.733	0.579	1.000	

<u>Depth 2 (Logistic Regression)</u>															
		Predicted													
		1 (-3m)	2 (-1m)	3 (1m)	4 (3m)	5 (5m)	6 (10m)	Σ							
Actual	1 (-3m)	6	2	3	0	0	1	12	Gradient location	AUC	CA	F1	Precision	Recall	
	2 (-1m)	4	3	2	0	3	0	12		-3 m	0.934	0.845	0.522	0.545	0.500
	3 (1m)	1	4	3	1	3	0	12		-1 m	0.589	0.775	0.273	0.300	0.250
	4 (3m)	0	0	6	4	2	0	12		1 m	0.581	0.662	0.200	0.167	0.250
	5 (5m)	0	1	4	2	5	0	12		3 m	0.867	0.845	0.421	0.571	0.333
	6 (10m)	0	0	0	0	0	11	11		5 m	0.794	0.789	0.400	0.385	0.417
Σ		11	10	18	7	13	12	71	10 m	1.000	0.986	0.957	0.917	1.000	

Figure 4.25: Discriminant analysis of gradient locations based on the **bacterial taxonomic diversity** structure. Confusion matrices and prediction quality parameters (see Methodology).

<b><u>Depth 1 (Neural Network)</u></b> Predicted 1 (-3m) 2 (-1m) 3 (1m) 4 (3m) 5 (5m) 6 (10m) $\Sigma$ Actual 1 (-3m) 3 0 2 3 2 2 12 2 (-1m) 1 5 3 2 1 0 12 3 (1m) 1 7 0 1 2 1 12 4 (3m) 1 2 3 1 4 1 12 5 (5m) 2 2 0 5 2 1 12 6 (10m) 0 5 2 1 0 4 12 $\Sigma$ 8 21 10 13 11 9 72								<b>Gradient location</b> <i>AUC</i> <i>CA</i> <i>F1</i> <i>Precision</i> <i>Recall</i> -3 m 0.360 0.806 0.300 0.375 0.250 -1 m 0.630 0.681 0.303 0.238 0.417 1 m 0.425 0.694 0.000 0.000 0.000 3 m 0.580 0.681 0.080 0.077 0.083 5 m 0.660 0.736 0.174 0.182 0.167 10 m 0.640 0.819 0.738 0.818 0.750					
<b><u>Depth 1 (Logistic Regression)</u></b> Predicted 1 (-3m) 2 (-1m) 3 (1m) 4 (3m) 5 (5m) 6 (10m) $\Sigma$ Actual 1 (-3m) 6 1 1 3 0 1 12 2 (-1m) 1 5 4 1 1 0 12 3 (1m) 0 3 6 3 0 0 12 4 (3m) 0 0 4 7 1 0 12 5 (5m) 1 1 0 4 5 1 12 6 (10m) 0 0 2 0 1 9 12 $\Sigma$ 8 10 17 18 8 11 72								<b>Gradient location</b> <i>AUC</i> <i>CA</i> <i>F1</i> <i>Precision</i> <i>Recall</i> -3 m 0.940 0.889 0.600 0.750 0.500 -1 m 0.865 0.833 0.455 0.500 0.417 1 m 0.805 0.764 0.414 0.353 0.500 3 m 0.790 0.778 0.467 0.389 0.583 5 m 0.800 0.861 0.500 0.625 0.417 10 m 0.960 0.931 0.783 0.818 0.750					
<b><u>Depth 2 (Neural Network)</u></b> Predicted 1 (-3m) 2 (-1m) 3 (1m) 4 (3m) 5 (5m) 6 (10m) $\Sigma$ Actual 1 (-3m) 3 0 2 3 2 2 12 2 (-1m) 1 5 3 2 1 0 12 3 (1m) 1 7 0 1 2 1 12 4 (3m) 1 2 3 1 4 1 12 5 (5m) 2 2 0 5 2 1 12 6 (10m) 0 5 2 1 0 4 12 $\Sigma$ 8 21 10 13 11 9 72								<b>Gradient location</b> <i>AUC</i> <i>CA</i> <i>F1</i> <i>Precision</i> <i>Recall</i> -3 m 0.542 0.764 0.370 0.333 0.417 -1 m 0.790 0.778 0.385 0.357 0.417 1 m 0.450 0.764 0.261 0.273 0.250 3 m 0.365 0.736 0.000 0.000 0.000 5 m 0.615 0.722 0.231 0.214 0.250 10 m 0.855 0.847 0.522 0.545 0.500					
<b><u>Depth 2 (Logistic Regression)</u></b> Predicted 1 (-3m) 2 (-1m) 3 (1m) 4 (3m) 5 (5m) 6 (10m) $\Sigma$ Actual 1 (-3m) 6 1 1 3 0 1 12 2 (-1m) 1 5 4 1 1 0 12 3 (1m) 0 3 6 3 0 0 12 4 (3m) 0 0 4 7 1 0 12 5 (5m) 1 1 0 4 5 1 12 6 (10m) 0 0 2 0 1 9 12 $\Sigma$ 8 10 17 18 8 11 72								<b>Gradient location</b> <i>AUC</i> <i>CA</i> <i>F1</i> <i>Precision</i> <i>Recall</i> -3 m 0.790 0.861 0.444 0.667 0.333 -1 m 0.705 0.764 0.261 0.273 0.250 1 m 0.285 0.639 0.071 0.062 0.083 3 m 0.480 0.750 0.182 0.200 0.167 5 m 0.880 0.819 0.581 0.474 0.750 10 m 0.790 0.806 0.364 0.400 0.333					

Figure 4.26: Discriminant analysis of gradient locations based on the **fungal taxonomic diversity** structure. Confusion matrices and prediction quality parameters (see Methodology).

<u>Depth 1 (Neural Network)</u>															
<div>Predicted</div> <div>1 (-3m)2 (-1m)3 (1m)4 (3m)5 (5m)6 (10m)Σ</div>										Gradient location	AUC	CA	F1	Precision	Recall
Actual	1 (-3m)	2	3	2	0	4	1	12	-3 m	0.705	0.806	0.222	0.333	0.167	
	2 (-1m)	1	3	6	0	1	1	12	-1 m	0.835	0.764	0.261	0.273	0.250	
	3 (1m)	0	4	4	1	3	0	12	1 m	0.700	0.694	0.267	0.222	0.333	
	4 (3m)	1	0	4	3	4	0	12	3 m	0.675	0.778	0.273	0.300	0.167	
	5 (5m)	2	1	2	5	2	0	12	5 m	0.535	0.694	0.154	0.143	0.167	
	6 (10m)	0	0	0	1	0	11	12	10 m	0.970	0.958	0.880	0.846	0.917	
	Σ	6	11	18	10	14	13	72							

Depth 1 (Logistic Regression)															
Predicted  1 (-3m)2 (-1m)3 (1m)4 (3m)5 (5m)6 (10m)Σ										Gradient location	AUC	CA	F1	Precision	Recall
Actual	1 (-3m)	3	3	4	0	1	1	12	-3 m	0.830	0.833	0.333	0.500	0.250	
2 (-1m)	2	4	5	1	0	0	12	-1 m	0.750	0.778	0.333	0.333	0.333		
3 (1m)	0	3	4	3	2	0	12	1 m	0.600	0.653	0.242	0.190	0.333		
4 (3m)	0	0	6	2	4	0	12	3 m	0.705	0.778	0.200	0.250	0.167		
5 (5m)	1	2	2	2	5	0	12	5 m	0.680	0.792	0.400	0.385	0.417		
6 (10m)	0	0	0	0	1	11	12	10 m	0.980	0.972	0.917	0.917	0.917		
Σ	6	12	21	8	13	12	72								
Depth 2 (Neural Network)															
Predicted  1 (-3m)2 (-1m)3 (1m)4 (3m)5 (5m)6 (10m)Σ										Gradient location	AUC	CA	F1	Precision	Recall
Actual	1 (-3m)	6	2	3	0	1	0	12	-3 m	0.789	0.887	0.600	0.750	0.500	
2 (-1m)	2	4	2	1	3	0	12	-1 m	0.653	0.761	0.320	0.308	0.333		
3 (1m)	0	3	3	3	3	0	12	1 m	0.546	0.690	0.214	0.188	0.250		
4 (3m)	0	2	5	2	3	0	12	3 m	0.647	0.746	0.182	0.200	0.167		
5 (5m)	0	2	3	4	3	0	12	5 m	0.738	0.732	0.240	0.231	0.250		
6 (10m)	0	0	0	0	0	11	11	10 m	1.000	1.000	1.000	1.000	1.000		
Σ	8	13	16	10	13	11	71								
Depth 2 (Logistic Regression)															
Predicted  1 (-3m)2 (-1m)3 (1m)4 (3m)5 (5m)6 (10m)Σ										Gradient location	AUC	CA	F1	Precision	Recall
Actual	1 (-3m)	6	2	2	1	0	1	12	-3 m	0.863	0.831	0.500	0.500	0.500	
2 (-1m)	3	2	5	1	1	0	12	-1 m	0.601	0.761	0.190	0.222	0.333		
3 (1m)	3	1	4	4	0	0	12	1 m	0.579	0.662	0.250	0.200	0.333		
4 (3m)	0	3	4	4	1	0	12	3 m	0.624	0.789	0.348	0.364	0.333		
5 (5m)	0	1	4	1	5	1	12	5 m	0.864	0.873	0.526	0.714	0.417		
6 (10m)	0	0	1	0	0	10	11	10 m	0.960	0.958	0.870	0.833	0.909		
Σ	12	9	20	11	7	12	71								

Figure 4.27: Discriminant analysis of gradient locations based on the PICRUST based **inferred functional diversity structure**. Confusion matrices and prediction quality parameters.



#### 4.4.2 Relationship between biotic and soil abiotic diversities

##### *Relationship between bacterial diversity and soil abiotic parameters*

Principal Coordinate Analyses (PCoA) illustrate the relationship between soil abiotic parameters and bacterial taxonomic diversity at the class taxonomic level.

[Figure 4.28](#), through 4.33 describe the diversity and relationships between bacterial communities and soil's abiotic parameters across sites and management gradients.

A main observation is that the bacterial community for the innermost *AgField* location (10m) clustered separately from the other gradient locations, irrespective of depths in all the sites ([Figure 4.28](#) to 4.31). Actinobacteria was always associated with this trend.

The separation between the outermost (*NatField*, -3m) and innermost (*AgField* 10m) locations on the gradient is clearly observable for all sites ([Figure 4.32](#), and 4.33) and, moreover, there is a closer association among the 10m samples for all sites. This confirms that the bacterial populations are more closely similar among themselves at 10m than at -3m.

The abiotic parameters follow the general trends described in the first part of the results section.

Most macronutrients and micronutrients associated directly with the 10m locations; Na, an indicator of manure addition is in larger proportions inside the *AgField*.

*Delta*- and *Beta-Proteobacteria* with *Nitrospira* and *Acidobacteriia* associate primarily with the -3m locations. The same *NatField* locations have more TOC. It is interesting to note that TN in the top layer, i.e. Depth 1, is larger in *NatField*, but for the Depth 2 the trend is reversed. This is likely an indication of the surface accumulation of OM in *NatField*, and of the more uniform distribution with depth of the OM in the *AgField*, possibly a result of tillage.

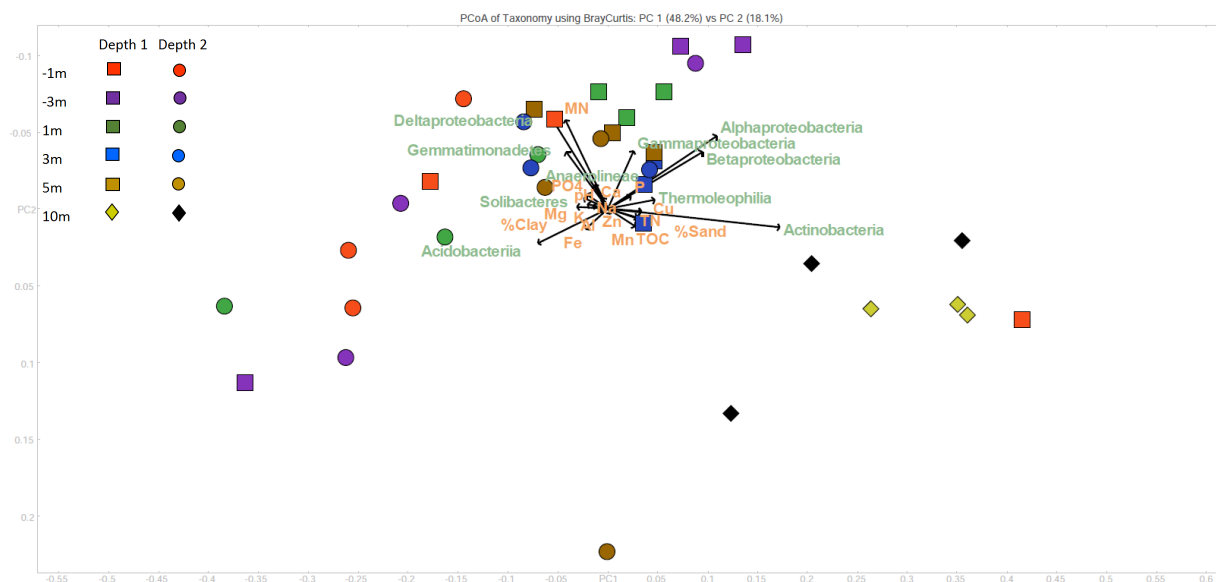


Figure 4.28: Relationship between bacterial taxa, (class level) and abiotic parameters. East sampling site.

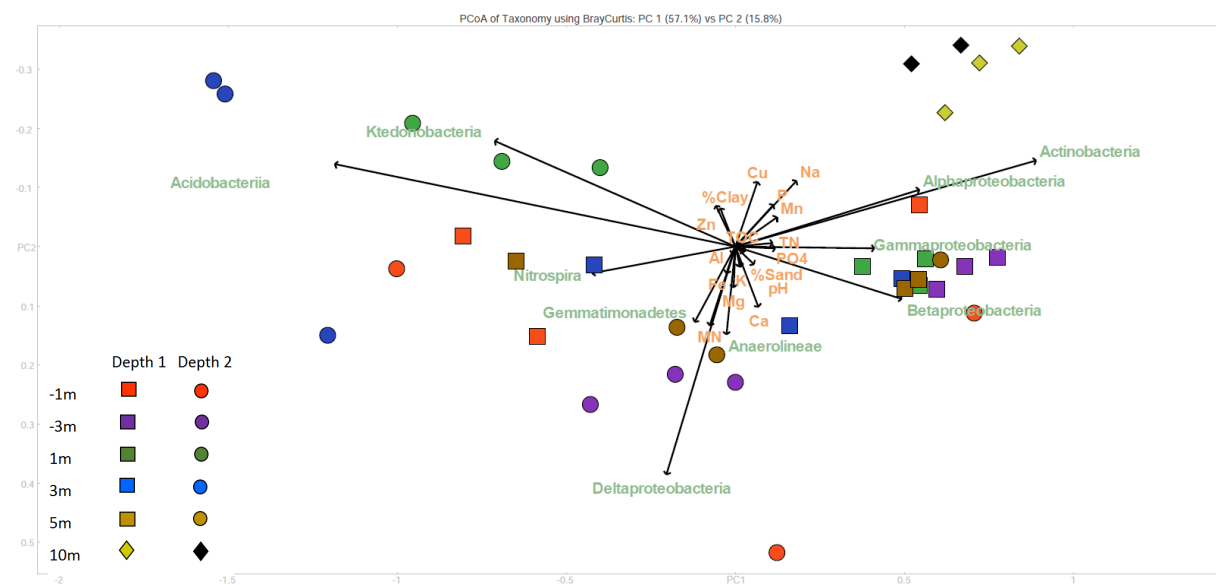


Figure 4.29: Relationship between bacterial taxa, (class level) and abiotic parameters. North sampling site.

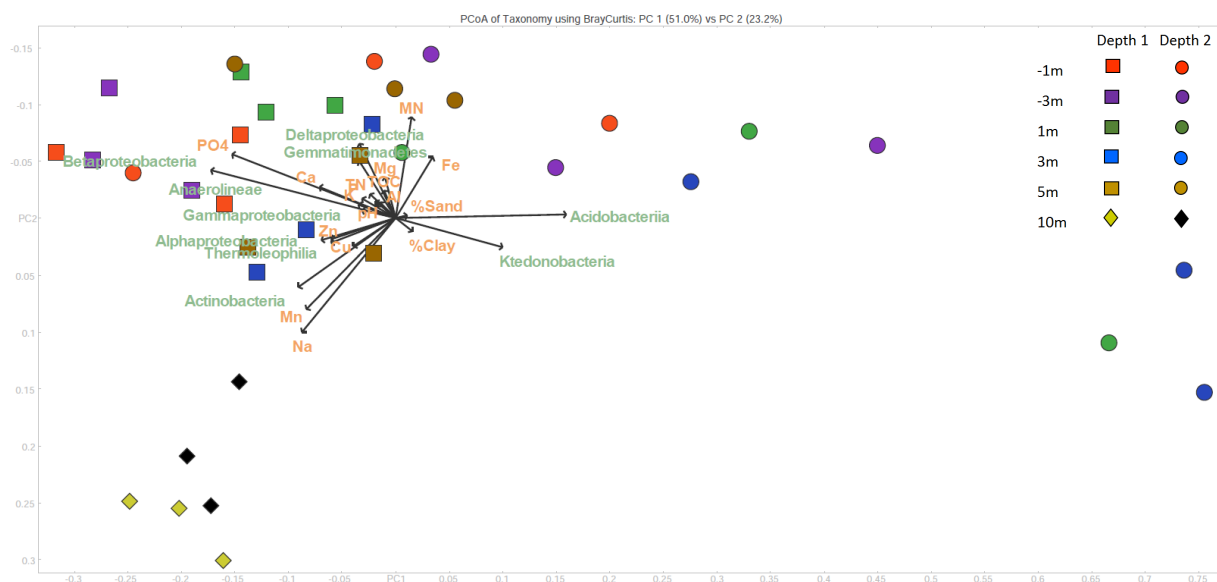


Figure 4.30: Relationship between bacterial taxa, (class level) and abiotic parameters. South sampling site.

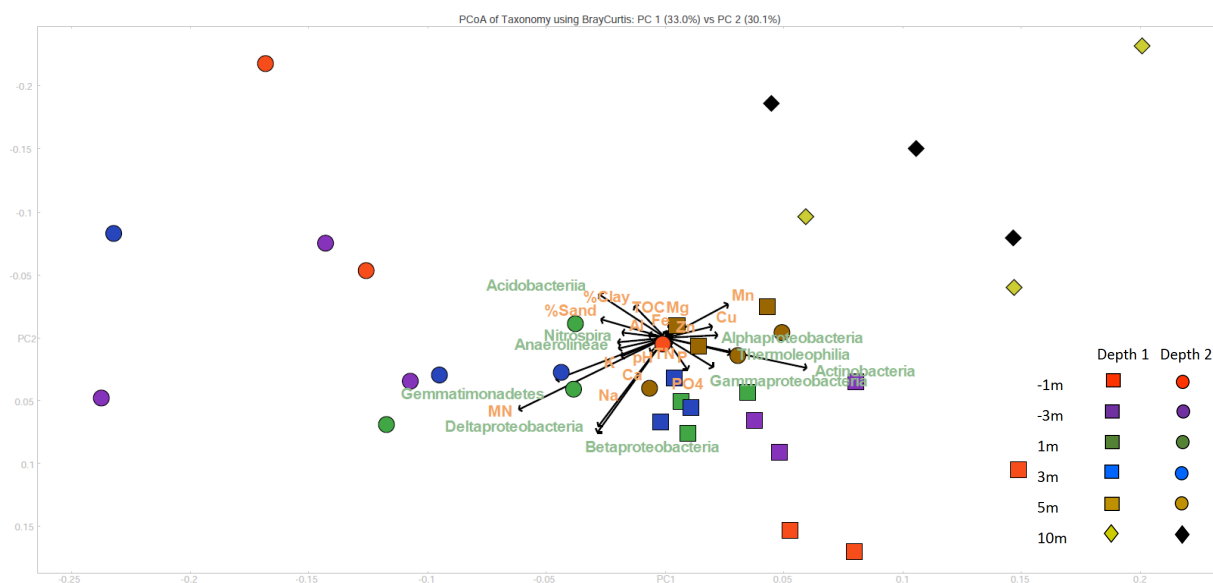


Figure 4.31: Relationship between bacterial taxa, (class level) and abiotic parameters. West sampling site.

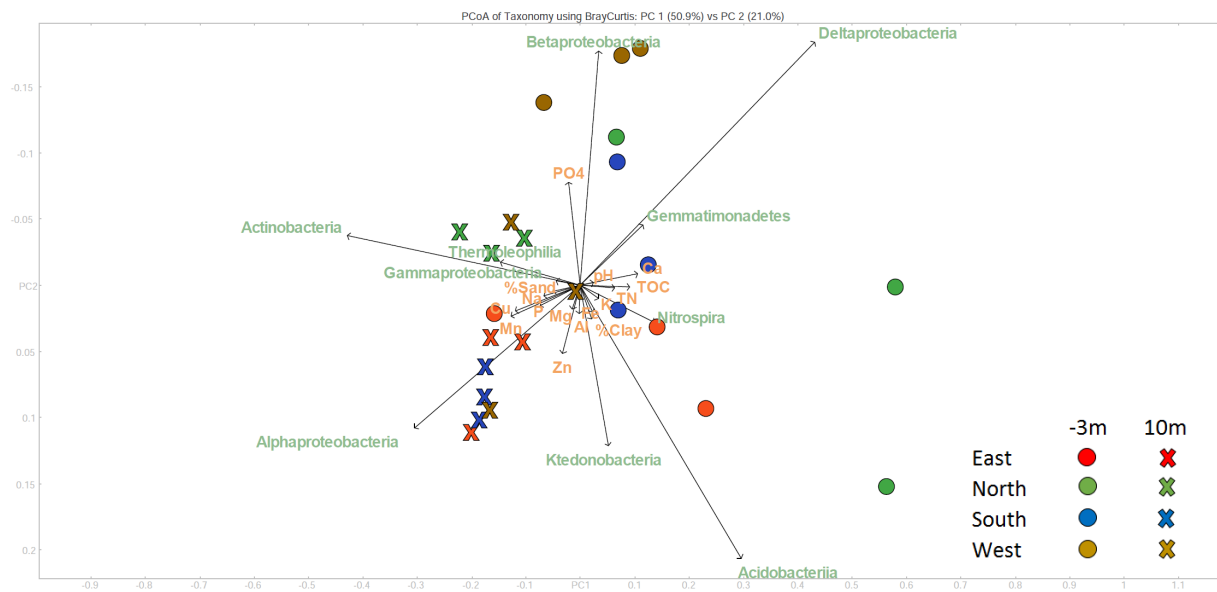


Figure 4.32: Relationship between bacterial taxa, (class level) and abiotic parameters. Comparing *AgField* (10m) and *NatField* (-3m) at Depth 1 sample points

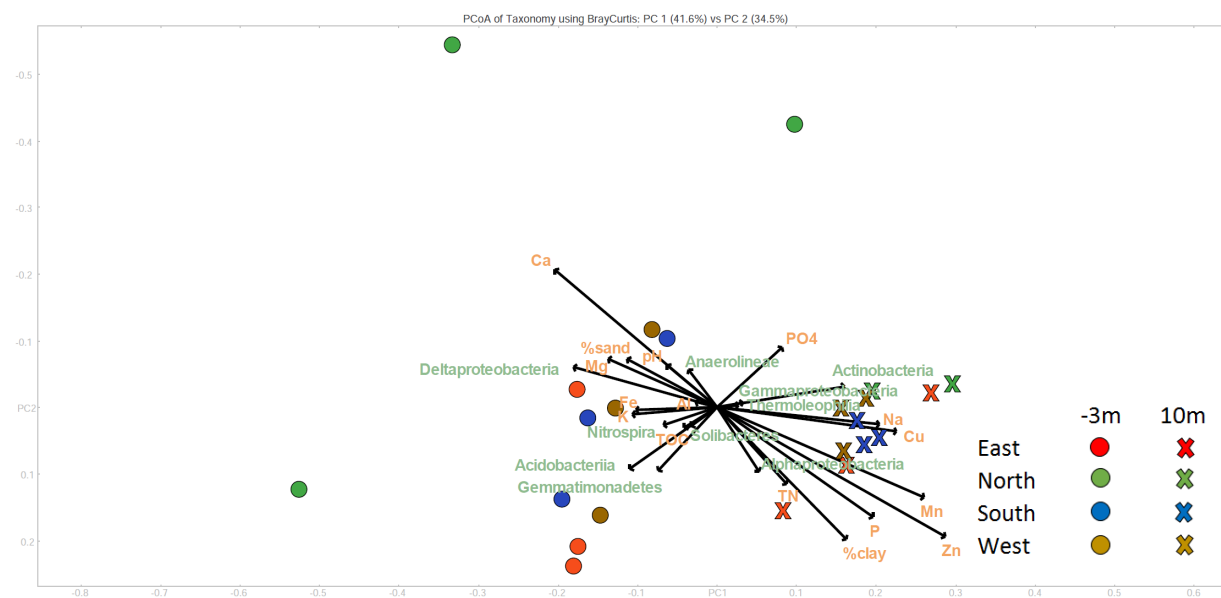


Figure 4.33: Relationship between bacterial taxa, (class level) and abiotic parameters. Comparing *AgField* (10m) and *NatField* (-3m) at Depth 2 sample points.

### *Relationship between fungal diversity and soil abiotic parameters*

Principal Coordinate Analysis, PCoA illustrate the relationship between abiotic Parameters and Fungal taxonomic diversity at Class Level.

The fungal community at the *AgField* (10m) are clustered irrespective with depths in all the sites even though the clustering of fungal community is not as strong as bacterial community.

Micronutrients such as Mn, Cu and Zn correlate with the *AgField* (10m) (see [Figure 4.38](#), and [Figure 4.39](#)).

There is no strong distinction between *AgField* and *NatField* at both depths, clearly less than what was noted for bacteria. This is likely due to the capacity of fungal hyphae to travel across larger distances than bacteria, towards the nutrients rich environment/ favorable condition.

Form

Delet

Delet

Form

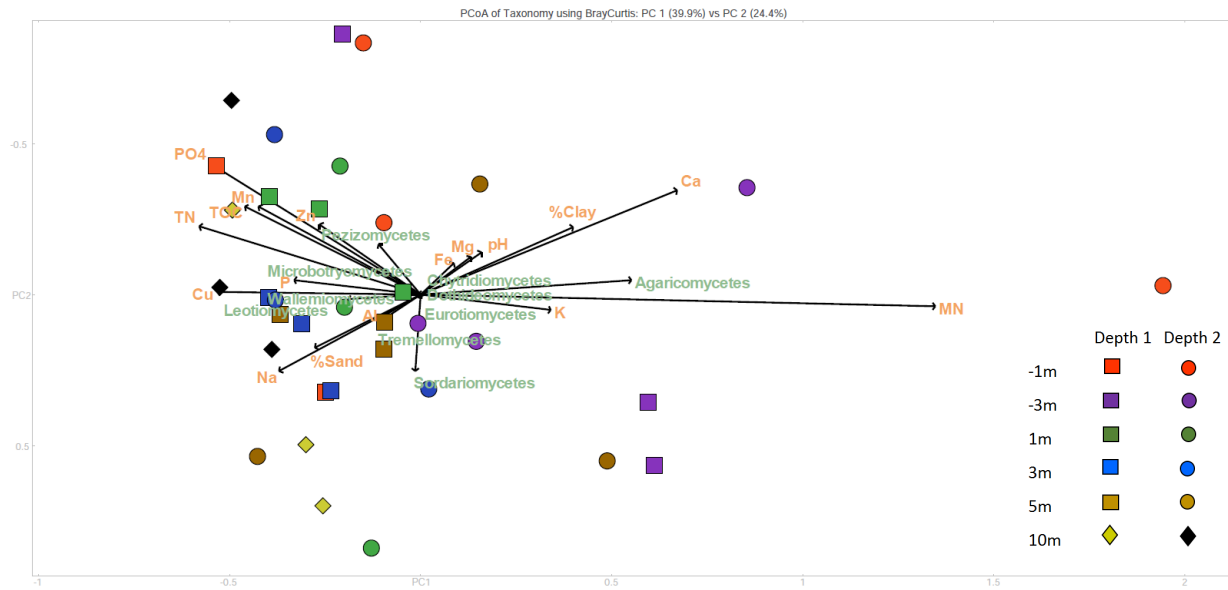


Figure 4.34: Relationship between fungal taxa, (class level) and abiotic parameters. East sampling site.

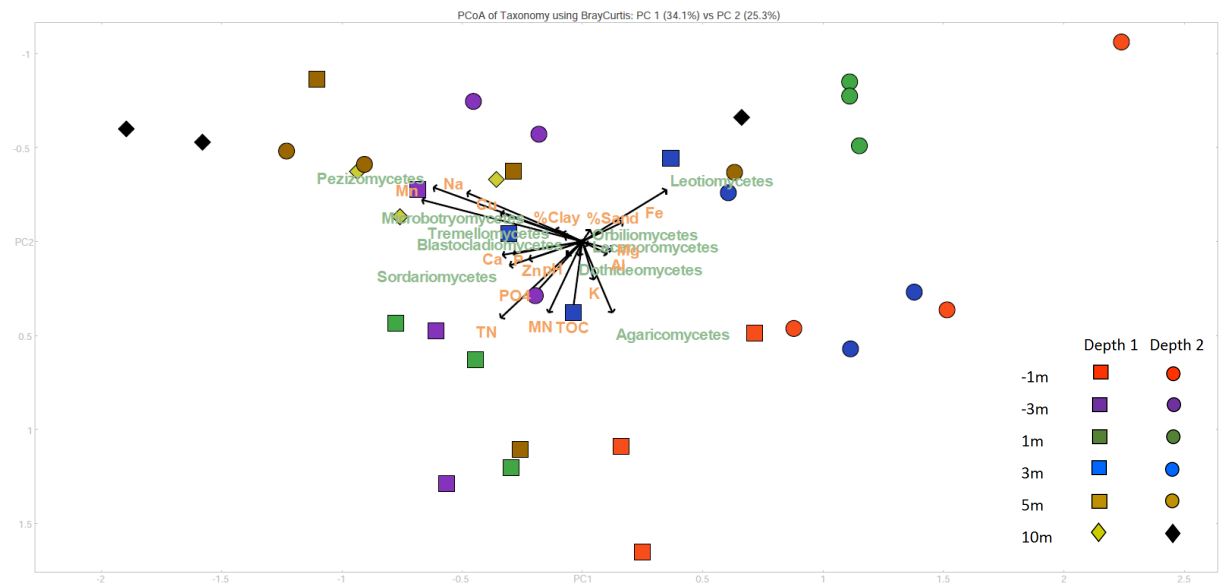


Figure 4.35: Relationship between fungal taxa, (class level) and abiotic parameters. North sampling site.

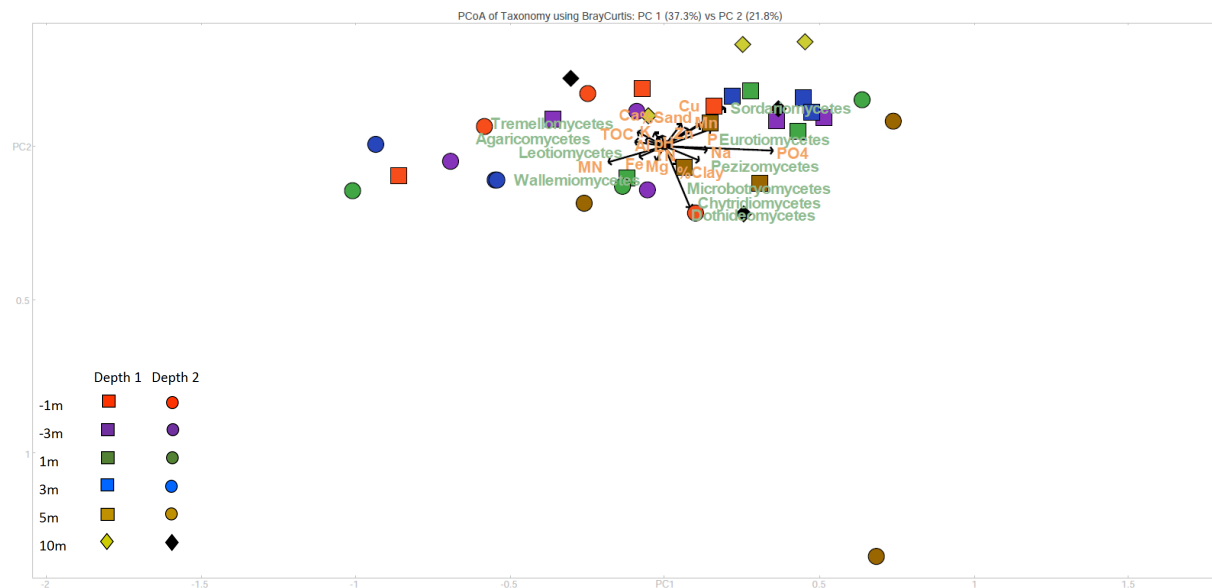


Figure 4.36: Relationship between fungal taxa, (class level) and abiotic parameters. South sampling site.

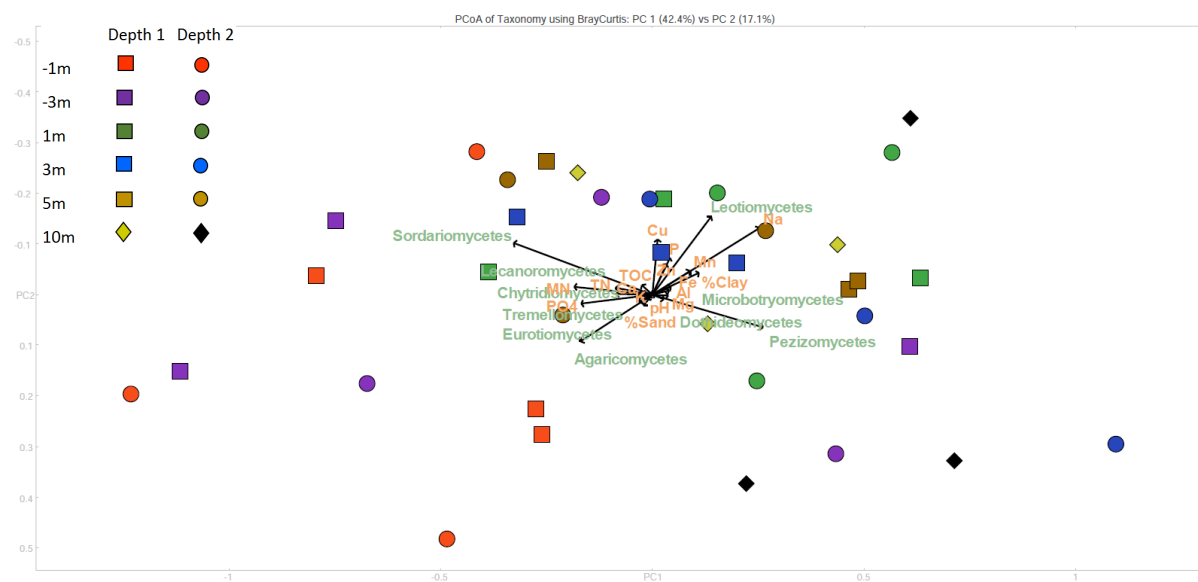


Figure 4.37: Relationship between fungal taxa, (class level) and abiotic parameters. West sampling site.





## 4.5 Functional Diversity

### 4.5.1 Metabolic Diversity

Results:

Principal Component Analysis (PCA) illustrating the Bacterial Metabolic diversity across sampling location in all sites. At Depth 1, PC1 and PC2 explained 78.00% and 18.00% of variance respectively. At Depth 2, PC1 and PC2 explained 92.00% and 6.00% of variance respectively ([Figure 4.40](#)).

The PCA results of Bacterial Metabolic Diversity in *AgField* (10m), highly managed field in all the sites colonized together illustrates that metabolic function at highly managed field is distinctive compared to the other management intensities, which is stronger at Depth 2 (less managed) ([Figure 4.41](#)).

Double Hierarchical Dendrogram Heat Map illustrating the Bacterial Metabolic diversity across sampling location in all sites at Depth 1 and Depth 2. X-axis represents the metabolic functions and the Y-axis represent the Sampling Location and Site. The relative abundance at Depth 1 is between -4.89 and 6.51 whereas in Depth 2, it is between -3.67 and 5.46 ([Figure 4.41](#)).

The Double Hierarchical Dendrogram Heat Map results of Bacterial Metabolic Diversity in *AgField* (10m), highly managed field in all the sites are dense which illustrates the metabolic function at highly managed field is distinctive compared to the *NatField* where they have different Bacterial Metabolic Diversity, which is mostly similar at both the depths.

In all site and at both the depths, amino acids such as cysteine, methionine, tyrosine, valine, leucine, isoleucine, arginine and proline and biosynthesis of secondary metabolites such as Butirosin and neomycin, Betalain, caffeine, clavulanic acid were found to increase when moving from natural to *AgField* whereas amino acids such as alanine, aspartate, glutamate and Phenylalanine and degradation such as lysine decreases when moving from natural to *AgField* (Appendix 9.1).

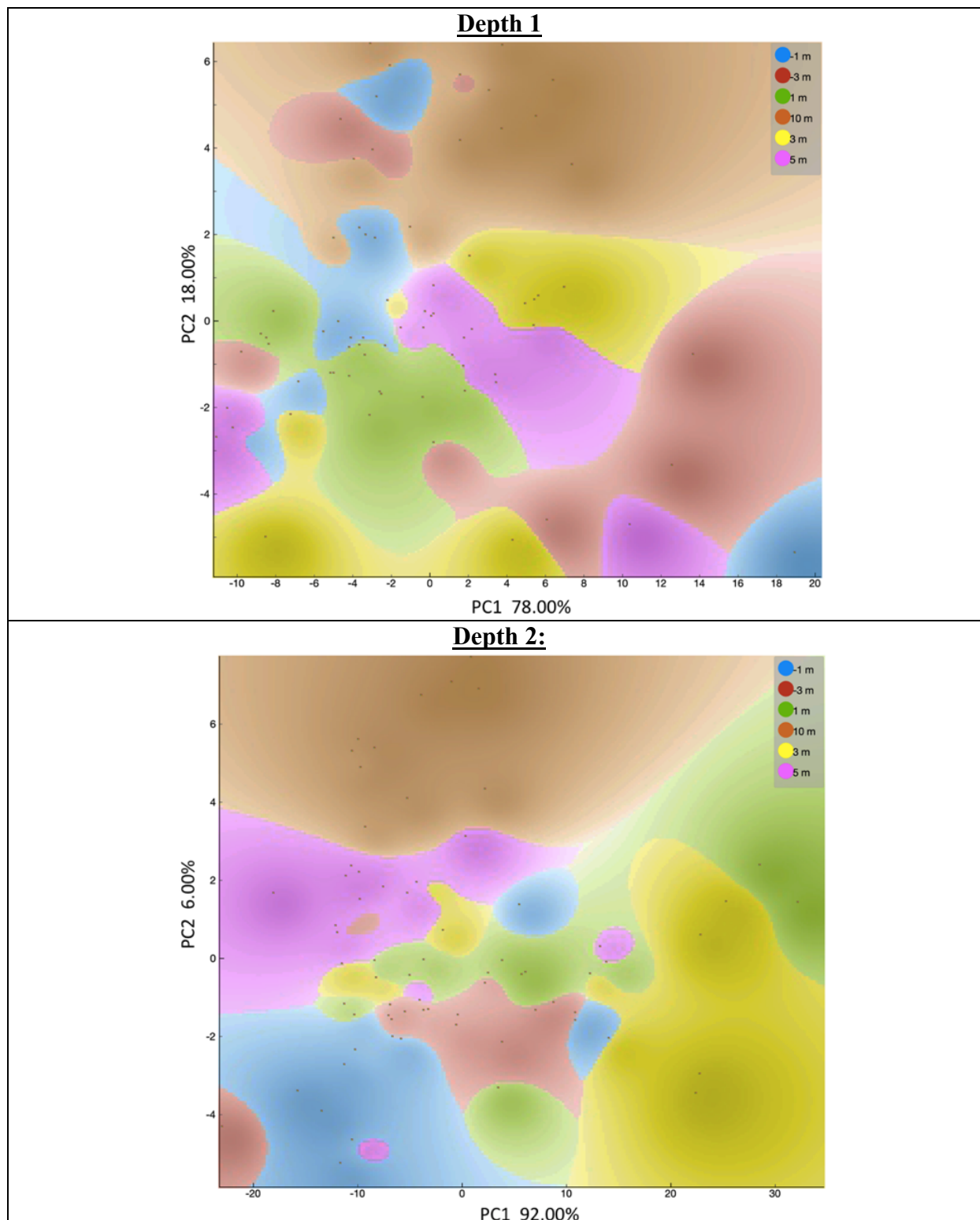


Figure 4.40: Relationship between Bacterial Metabolic diversity across Management Intensity in all sampling sites at Depth 1 and Depth 2.





#### 4.5.2 Xenobiotics Biodegradation

##### Results:

Principal Component Analysis(PCA) illustrating the bacterial biodegradation of Xenobiotic across sampling location in all sites. At Depth 1, PC1 and PC2 explained 70.00% and 19.00% of variance respectively and at Depth 2, PC1 and PC2 explained 59.00% and 32.00% of variance respectively ([Figure 4.42](#)).

The PCA results of bacterial biodegradation of xenobiotic in Agricultural field (10m), highly managed field in all the sites colonized together illustrates that metabolic function at highly managed field is distinctive compared to the other management intensities, which is stronger at Depth 2 (less managed) ([Figure 4.43](#)).

Double Hierarchical Dendogram Heat Map illustrating the bacterial biodegradation of xenobiotic across sampling location and sites at Depth 1 and at Depth 2. X-axis represent the bacterial biodegradation of xenobiotic and the Y-axis represent the Sampling Location and Site. The relative abundance at Depth 1 is between -4.23 and 3.40 whereas in Depth 2, it is between -2.85 and 3.28 ([Figure 4.43](#)).

The Double Hierarchical Dendogram Heat Map results for bacterial biodegradation of xenobiotics in *AgField* (10m), highly managed field in all the sites are dense illustrates that metabolic function at highly managed field is distinctive compared to the *NatField* where they have different bacterial xenobiotics biodegradation, which is mostly similar at both the depths ([Figure 4.43](#)).

Bacterial biodegradation of xenobiotics is dominant at *AgField* (highly managed field, 10m). Such metabolism is similar in both the depths.

At 10m (*AgField*), caprolactam degradation, benzoate degradation, ethylbenzene degradation, polycyclic aromatic hydrocarbon degradation, bisphenol degradation, naphthalene degradation, amino benzoate degradation, dioxin degradation, styrene degradation, fluorobenzoate degradation, chlorocyclohexane and chlorobenzene degradation and chloroalkane and chloroalkene degradation, toluene degradation, drug metabolism- cytochrome P450, metabolism of xenobiotics by cytochrome P450 in both the depths of East and North site. Above all at Depth 2 of all sites, 1,1,1-Trichloro-2,2-bis(4-chlorophenyl) ethane(DDT) degradation increase from *NatField* to *AgField* (Appendix 9.2).

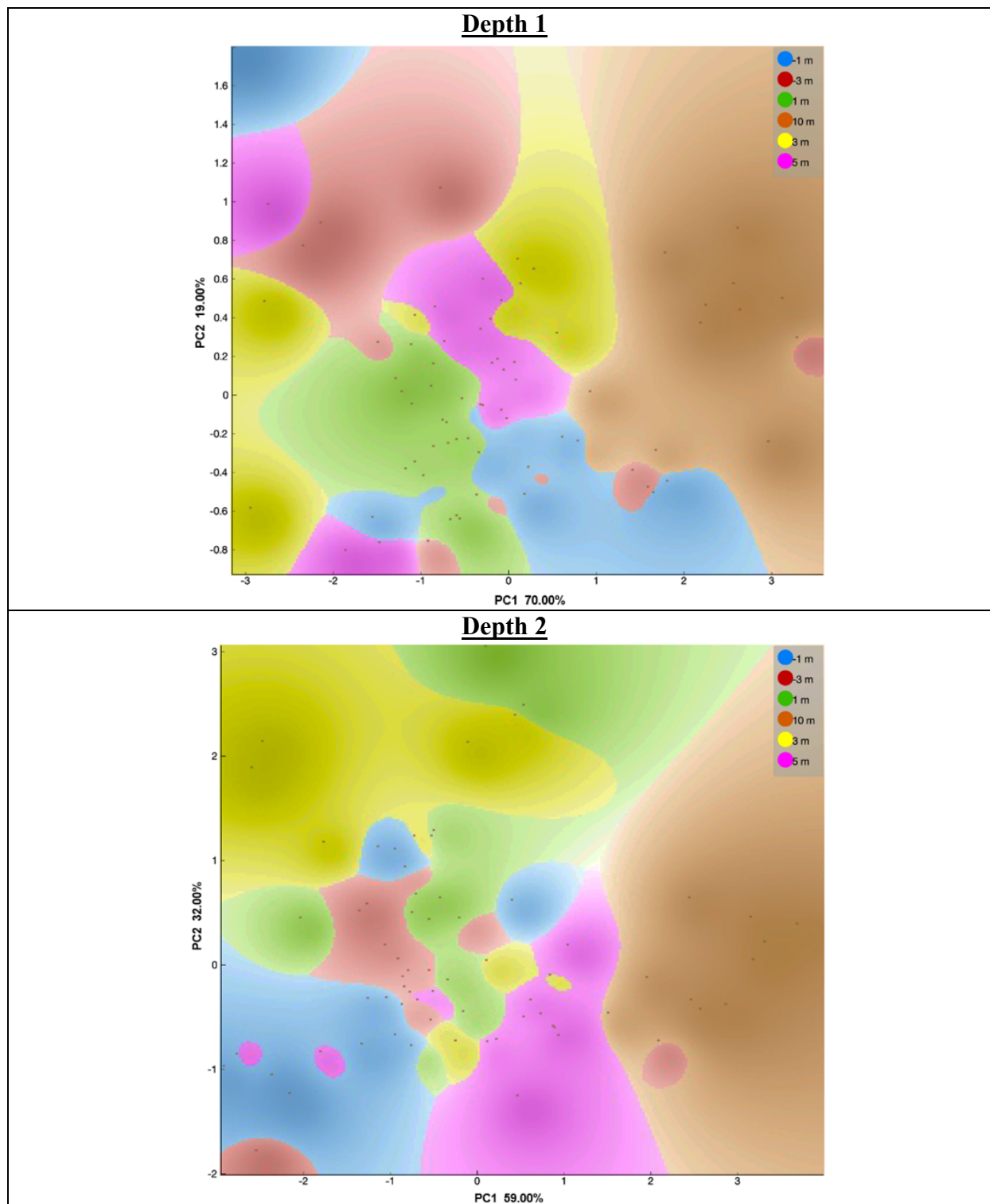


Figure 4.42: Relationship between Bacterial Biodegradation of Xenobiotic across Management Intensity in all sites at Depth 1 and Depth 2.



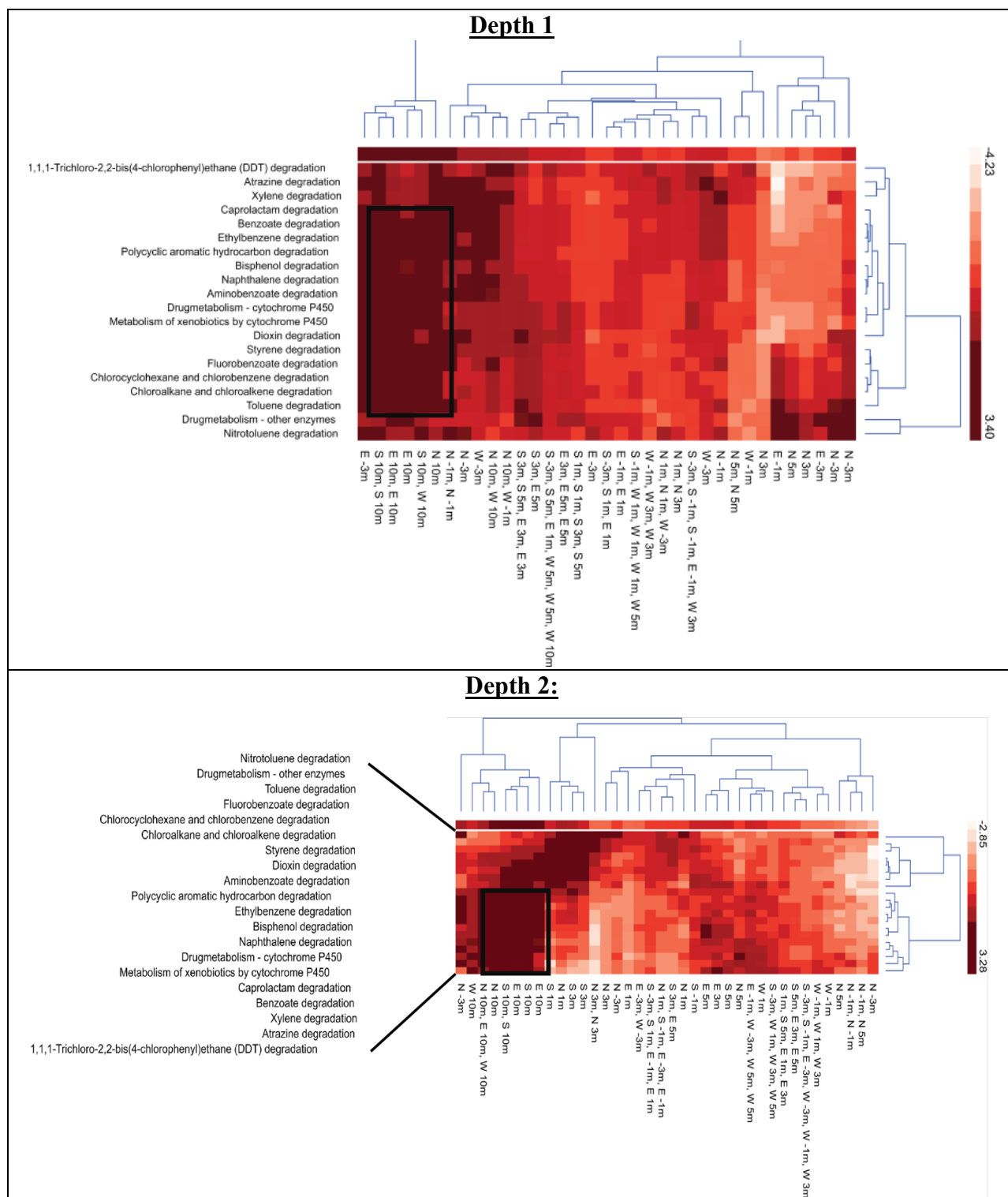


Figure 4.43: Relationship between the Bacterial Biodegradation of Xenobiotic Biodegradation across Management Intensity in all sites at Depth 1 and Depth 2.

## 5 Discussion

### *Physical Parameters:*

Natural and tillage erosion do affect soil texture along slopes ([Figure 4.2](#), and [Figure 4.3](#)). Tillage is known to accelerate erosion on slopes, leading to transfer of smaller particles, e.g. clay, down gradient. Moreover, podzols are known to naturally have coarser textures in the top horizon(s). Analyses of the Cormack test site have confirmed that the top layer, i.e. Depth 1, was enriched in the sand fraction at the East, North and South sites. Changes along slope, expectedly most obvious for the steeper East site, also confirmed the role of downslope erosion in controlling texture. Note that the more eroded East site also has larger aluminium and iron concentrations, a likely result of the stripping of clays and thus intrinsic increase in the metal oxides more resilient to erosion. On the other hand, the nearly flat West site has shown little difference in texture along the sampled gradient, a further confirmation of the governing role of the slope in defining texture for the test sites.

### *Chemical Parameters:*

Exceedingly low pH values in *AgField* are deleterious to plant growth and thus acidic soils do regularly receive carbonate amendments, commonly limestone, with a goal to increase the pH at or nearly a neutral state. It is unclear if large applications of organic matter through the application of manure, may lead to an increase or decrease in pH; significant leaching of nitrate and addition of ammonium based nitrogen fertilizers are known to acidify soils. The *AgField* receives manure regularly, often at least twice a year, and also has historically received, limestone amendments as recommended. However, none of these applications are consistently recorded in writing. Moreover, urea and ammonium nitrate are also commonly applied.

Several parameters point to the fact that *AgField* receives sufficient manure to modify soil's chemistry. Tests have shown that pH within the *AgField* boundaries to be significantly lower than for *NatField*, although the difference was within <0.5 pH units; nevertheless, this decrease was consistent across the four tested gradients. If this trend is consistent across other soils in the province, then such a trend might need to be assessed in the context of the current practices. It is also known that manure application increases the total and available phosphorus content in soil

Delet  
Delet  
Form  
Form



(Oloo et al., 2016) much of which can be chemically fixed by the free aluminum and iron common in acid soils. For the *AgField* total and available phosphorus is higher for the Depth 1.

Extensively leached boreal podzols do not accumulate sodium naturally. However, livestock manure contains sodium, and thus one might assume that any significant amount of sodium in a manured, managed podzol would originate in the applied livestock manure (Chang et al., 1990). *AgField* had a statistically significant higher concentration of sodium in soil (mean = 360.7) whereas the Natural field had significantly lower concentration of sodium (mean = 65.8) (see Appendix 7.4.2). Depth 1 and Depth 2 had no significant difference (Appendix 7.4.3). Copper and zinc are also commonly found in livestock manure and thus expected to be found in higher concentrations in manured soils (Hokayem and Azzi 2014; Ginzburg 1960); *Agfield* had higher concentrations of both Cu and Zn. All these measurements point to the impact on manure on the *AgField* chemistry.

Depth 1 had significantly more TN and TOC than Depth 2 irrespective of *Agfield* and *Natfield* (see Appendix 7.16.3). This confirms the preferential accumulation of recently added organic matter and decaying plant material in the top soil layer.

On the other hand, podsolization leads to mobilization of the organic matter in the topsoil and its accumulation as stable organic matter, in the form of organic and humic and fulvic acids, in the deeper soil layers, i.e. the B horizon (Mokma and Buurman, 1987). Same podsolization process also favours the release, and downward flow of aluminium and iron. This leads to the formation of a metal-humic/fulvic acids complexes in the top of the B horizon, mainly as bi and trivalent cation complexes. For example, at low pH (<3.5), the fulvic acids form organometallic complexes, in the order of  $\text{Fe}^{3+} > \text{Al}^{3+} > \text{Ca}^{2+} > \text{Mg}^{2+}$  whereas at pH 5.0, humic acids form complexes preferentially in order of  $\text{Al}^{3+} > \text{Fe}^{3+} > \text{Mg}^{2+} > \text{Ca}^{2+}$ . At the test plots, the East site has the lowest average pH (mean= 5.27) and North site has a higher pH (mean= 6.09) (see Appendix 7.3.1). As mentioned above *Natfield* had significantly higher pH (mean= 5.78) than *Agfield* (mean= 5.33) (Appendix 7.3.2). Depth 1 had significantly higher pH (mean= 5.66) than Depth 2 (mean= 5.55) (Appendix 7.3.3). Magnesium, aluminum and iron are higher at East site and lower at North site (Appendix 7.10.1, 7.11.1 and 7.12.1) whereas calcium is higher at North site, and lower at East site (Appendix 7.6.1). This pattern in the amount and availability of these cations is expectedly associated with the respective pH values across the sampled sites.

## Microbial Taxonomic Diversity:

### **Bacterial diversity:**

A greater bacterial diversity was measured in the *NatField* for both the depths (Figure 4.20). The richness and ecological diversity indices (Figure 4.20) have shown that for the *NatField* both the total bacterial abundance and also the proportion of rare bacterial taxa were larger than for *Agfield* (Figure 4.20). A direct comparison (i.e. bacterial beta diversity) with the outermost *NatField* gradient point (-3 m) has shown a decrease in similarity with the -3 m when progressing towards the centre of the *AgField*; this was true across all sites and both depths (Figure 4.22, and **Error! Reference source not found.**).

The proportional abundance of certain taxa decreased towards the center of the *AgField* while for other taxa increased their proportional abundance increased towards the center of the *AgField*. It is expected that these changes are a direct reflection of the changes in the abiotic conditions as affected by agricultural management (Figure 4.32, and Figure 4.33).

### **Bacteria abundance describing the Agricultural field:**

*Deinococcus- Thermus* phylum, *Acidobacteriia- Acidobacteria* phylum, *Thermomicrobia* phylum, *Thermoleophilia* class (*Actinobacteria* phylum), *Epsilonproteobacteria* class, *Holophagae* class of the genus *Geothrix*, and *Ktedonobacteria* were best described in *Agfield* (Appendix 8.1). These taxa cover a wide taxonomic range with a wide range of environmental requirements. For example, the *Deinococcus-Thermus* phylum, *Actinobacteria's Thermoleophilia* class, and the *Thermomicrobia* phylum are known to contain stress resilient organisms, many resistant to dry and hot systems (Crits-Christoph et al., 2013; Goodfellow, 2012; J. J. Lee et al., 2013; Sergio et al., 2011; Shvrlata and Satyanarayana, 2015). *Ktedonobacteria*, A class of *Chloroflexi* phylum, might also be similar to *thermobacteria* found in hot and acid environments.

It was thus of note that *Deinococcus- Thermus* phylum, while better represented within the *Agfield* they were best represented in the West site, i.e. the driest site in the test plot. This site is located at the highest point of the test plot and is the only site with no evidence of groundwater

within 2 m from the surface (a parallel research activity was carried at the same time, but do not describe in this thesis, involved digging of 2 m deep trenches along the gradients described here). This and the larger sand proportion suggests a soil consistently drier and thus expected to be warmer than the soil in all other sites. *Epsilonproteobacteria*, *Geothrix spp.*, and *Ktedonobacteria* class may point to anoxic, and acid conditions and may be associated to variability in reactive iron species (Acton, 2011; Fukunaga, 2014; Grote et al., 2007; Kim et al., 2015; Yabe et al., 2017). Accordingly, these taxa were better descriptors of the wetter North and East sites.

*Synergistia* class- *Synergistetes* phylum, *Actinobacteria* phylum and *Bacteroidia* class were also best describers of *Agfield* (Appendix 7.18). These taxa are well known to be associated with, manure application. For example, *Synergistia* class- *Synergistetes* phylum are known to found in gastrointestinal tract of animals (Jumas-bilak et al., 2014). *Actinobacteria* phylum are known to have direct impact with manure application and mediates decaying of plant debris and formation of organic carbon (Eisenlord et al., 2012; Piao et al., 2008). *Bacteroidia* class are known to found in rotting/ nutrient rich biomass (S. He et al., 2017; Lu and Zhang, 2014; Vladimir et al., 2015). Above all, some were best described in *Agfield* (Appendix 7.18). *Bacilli* class and *Clostridia* class of *Firmicutes* phylum are well known to involve in mineralization of organic compounds, pesticides and hydrocarbons (Garbeva et al., 2003; Siala et al., 1974).

### ***Bacterial abundance describing the Natural field:***

Flavobacteriia class of Bacteroidetes phylum, *Ignavibacteria* class of *Chlorobi* phylum, *Gemmatimonadetes* phylum and *Betaproteobacteria* class of *proteobacteria* phylum were best describers of *Natfield* (Appendix 7.18). These taxa are known to involve in nitrogen cycle. For example, *Flavobacteriia* class of *Bacteroidetes* phylum and *Ignavibacteria* class of *Chlorobi* phylum are known to involve in denitrification processes (Horn et al., n.d.; Zverlow.V et al., 2015). *Betaproteobacteria* class of *proteobacteria* phylum are known to involve in Ammonia Oxidation processes (Fierer et al., 2012; Lepleux et al., 2012; Martin et al., 2012; Prosser, 2012) which is confirmed with high TN content in *Natfield* (Appendix 7.16.2). *Mollicutes* class of *Tenericutes* phylum and *Chlorobi* phylum were best describers of *Natfield* (Appendix 7.18). These taxa are well known to be affected by land management practices which causes stress (Bertrand et al., 2011; Bryant and Liu, 2013; Canfora et al., 2014; Constancias et al., 2015).

### ***Fungal diversity:***

Fungal Alpha diversity, there is no clear shift in *NatField* and *AgField* at both the depths whereas fungal Beta diversity decreases when moving from *NatField* to *AgField* in all the sites at both the depths except at Depth 1 of East and North sites (Figure 4.21, Figure 4.23, and Table 4.2).

Studies have shown notable impacts of spatial distance on microbial community dissimilarity (King et al., 2010; Nunan et al., 2003, 2002).

### ***Fungal abundance describing the Agricultural field:***

*Ustilaginomycetes* and *Wallemiomycetes* class of *Basidiomycota* phylum, *Pezizomycetes* class of *Ascomycota* phylum and *Chytridiomycetes* class of *Chytridiomycota* phylum were best describers of *Agfield* (Appendix 8.2). This covers a wide range of taxa causing diseases to crop plants. For example, *Ustilaginomycetes* class of *Basidiomycota* phylum are known to cause smut disease (Fai and Grant, 2009; Renker et al., 2003) whereas *Wallemiomycetes* class of *Basidiomycota* phylum are known to cause mold in agricultural plants (Takahiko Nagano, n.d.; Zalar et al., 2005; Zenova et al., 2007). *Pezizomycetes* class of *Ascomycota* phylum are saprobic and rarely plant pathogen (Bell et al., 2014; Pfister.H, 2015; Tedersoo et al., 2013) *Chytridiomycetes* class of *Chytridiomycota* phylum are saprophytic and parasitic on plants (Agrios.N George, 2005; Carol et al., 2004; Glazovsky and Nina, 2009).

*Tremellomycetes* and *Agaricostilbomycetes* class of *Basidiomycota* phylum, *Monoblepharidomycetes* class of *Chytridiomycota* phylum were also best describers of *Agfield* (Appendix 7.19). These taxa are well known to be associated with manure application. For example, all of the above taxa are saprophytes, bio-degraders and can survive in nutrient rich environments degradation (Bauer et al., 2009; de Menezes et al., 2012; Grissa et al., 2010; Karpov et al., 2017; Liu et al., 2015; M. Catherine Atme David J. McLaughlin, 2014; Micheal Wess Jose Paulo Sampaio Robbert Bauer, 2014; Peter, 2014)

Apart from above taxa, few taxa are well known to be associated with low pH were also best describers of *Agfield*. For example, *Leotiomycetes* class of *Ascomycota* phylum and *Cystobasidiomycetes* class of *Basidiomycota* phylum are well known to found in low pH (<4.5) (Jasrotia et al., 2014; M. Catherine Atme, Merje Toome, 2014; Mueller et al., 2016; Tong et al.,

2017; Yurkov et al., 2015) which confirms with the low pH in *Agfield* (Appendix 7.3.2).

### ***Fungal abundance describing the Natural field:***

*Saccharomycetes* and *Lecanoromycetes* class of *Ascomycota* phylum and *Agaricomycetes* and *Dacrymycetes* class of *Basidiomycota* phylum were best descriptors of *Natfield* (Appendix 7.19). These cover a wide range of taxa with a wide range of natural environmental requirements. For example, *Saccharomycetes* and *Lecanoromycetes* class of *Ascomycota* phylum and *Agaricomycetes* and *Dacrymycetes* class of *Basidiomycota* phylum are well known to feed on forest tree, decomposer of tree debris and involves in lignin degradation (Aislabie et al., 2013; Arfi et al., 2012; Bester, 2005; Capriotti, 1955; Eldridge et al., 2015; Fai and Grant, 2009; Francis, 2014; Gueidan et al., 2014; Hibbett et al., 2007; Knight and Goddard, 2015; Mohammadi-Sichani et al., 2017; Nagy et al., 2016; Shirouzu et al., 2016; Steven et al., 2015; Tonouchi, 2009).

### **Microbial Functional Diversity:**

#### *Metabolic function except Xenobiotics:*

Amino acids and proteins are the major sources of organic nitrogen in soil and are readily available to microorganisms as the source of carbon, and nitrogen in soil (Vinolas et al., 2001). Soil abiotic parameters such as temperature and nutrient content affect the amino acid variability (Moe, 2013). Free amino acids (FAAs) (dissolved in soil solution due to absence of covalent bond) produced via protein depolymerisation using extracellular enzymes. Soil mainly consists of organic nitrogen and approximately 40% of total nitrogen present in the soil is in the form of proteins and peptides. Proteins are the largest, reliable source on FAAs in soil, and depolymerisation of protein nitrogen to amino acid nitrogen is the rate-limiting step in the nitrogen cycle of soil (Rennenberg et al., 2009; Schimel and Bennett, 2004; Schulten and Schnitzer, 1998). In soil, FAA is reduced due to mineralization to inorganic nitrogen via ammonification and nitrification, microbial immobilization via binding, plant uptake. Losses via leaching and adsorption to charged surface (Stevenson, 1982; Yu et al., 2010).

Amino acids such as cysteine, methionine, tyrosine, valine, leucine, isoleucine, arginine and proline are found to be higher in *AgField* (see Appendix 9.1). Amino acids found in *AgField* due

to their environmental condition, for example, amino acids such as alanine, cysteine, valine, methionine, isoleucine, leucine, tyrosine, and phenylalanine are reported to be high in fields growing wheat (Jämtgård, 2010; Sciences, 1981). Amino acids such as arginine and proline metabolism found to help in the degradation of aromatic carbon sources (Nikel.I, 2016). In plants, it acts as nitrogen storage (Gioseffi et al., 2012; Winter et al., 2015). Cysteine and methionine are found to come from crop plant debris (Gahan and Schmalenberger, 2016). Glycine, serine and threonine are found in the root exudates (Pearce et al., 1995). Secondary metabolites such as neomycin acts as carbon source in soil for microbes which is also resistance to antibiotics (Zhang and Dick, 2014).

#### *Metabolism of Xenobiotics:*

Metabolism of xenobiotics is found to be higher in *AgField* (Appendix 9.2). Xenobiotics such as caprolactam, toluene, bisphenol, ethylbenzene, benzoate, fluorobenzoate, amino benzoate and polycyclic aromatic hydrocarbon, naphthalene and styrene are mainly from anthropogenic activities. For example, caprolactam, toluene and bisphenol in soil mainly comes from nylon 6 resins and plastic bags. In soil, caprolactam degradation is very fast due to its low half-life (5-14 days) via microbial and chemical degradation processes (Howard, 1989). In case of leaching, it is expected to biodegrade under aerobic condition and chemical degradation whereas bisphenol is biodegraded under aerobic condition and chemical hydrolysis or volatilization was never noticed in soil (Howard, 1989). Even though there is no data available on the biodegradation of toluene, it is expected to biodegrade in soil due to the presence of amine group (toluene 2, 4- diamine) (Howard, 1989). Ethylbenzene, benzoate, fluorobenzoate, amino benzoate and polycyclic aromatic hydrocarbon is mainly come from petroleum and gasoline products. In soil, Ethylbenzene is rapidly evaporated into atmosphere. Ethylbenzene is mostly leached to groundwater whereas the SOC content is very low and its biodegradation is very slow after acclimation (Howard, 1989). Naphthalene mainly comes from crude oil, forest fires and petroleum refining and coal tar distillation which is mostly evaporated from the soil and its biodegradation is faster in surface soil and become slow with increase in depth (Howard, 1989). Styrene is mainly coming from automobile parts such as paints, waxes and metal cleaners. If biodegradation is high under sandy loam soil (87-95%) (Howard, 1989).

In response to increasing environmental and safety concerns, most uses of DDT in Canada were phased out by the mid-1970s. Registration of all uses of DDT was discontinued in 1985, with the understanding that existing stocks would be sold, used or disposed of by December 31, 1990. The presence of DDT degradation at Depth 2 and their degradation increases from *NatField* to *AgField* indicates that the DDT degradation is very slow in soil (“List of Toxic Substances Managed Under CEPA (Schedule 1)- Dichlorodiphenyltrichloroethane,” 2013).

## 6 Conclusions

Physicochemical properties are affected by management practices, natural slope and wet and dry nature of the field. Depth 1 is affected more than the Depth 2 as management practices are mostly on the surface soil. Liming practices are expected to increase the pH, but in our study, *AgField* has the lowest pH in all sites studied. This change might be caused by several processes such as cations removal by plant uptake, leaching, inorganic & organic fertilizers and chemical amendments, change in OM content, high water percolation and infiltration rates (USDA *NatField* Resources Conservation Service 2011).

Bacterial community in *NatField* and *AgField* are distinct due to the difference in the nutrient source, and adaptation and survival behaviors. Nutrients such as Na, Mn, Cu and Zn are in *AgField* at both the depths which likely correlates to manure applications. Fungal community is not much distinct in *NatField* or in *AgField*. Hyphal movement in search of nutrient from the nutrient deficit place to nutrient rich place makes it spread all over the field.

Some can live in/on both plants and trees and some are myco parasites make it survive in both *NatField* and *AgField*.

*AgField* is found to have distinct metabolic (amino acids, carbohydrates, lipids, glycan, vitamins and co-factors) and xenobiotic metabolism. This is mainly due to comparatively high crop plant degradation in *AgField* which is the primary source of lipids, amino acids and carbohydrates. Fungi and actinomycetes are the primary producers of secondary metabolism. Xenobiotics such as Atrazine and DDT in *AgField* mainly comes from management practices to control weeds and insects.

It is found that, while the natural conditions tested have distinct taxonomic and functional diversities, they become increasingly similar towards the center of the *AgField*, away from the natural edge. It is found that Depth 1 and Depth 2 has distinct taxonomic and functional diversity. Depth 1 has more distinct abiotic factors than Depth 2 towards the center of the field from the natural edges. Thus, these results support both of the hypotheses that “*Land management affects the taxonomic and functional diversity of microorganisms*” and also that “*the shift in taxonomic and functional diversity is directly related to the distance from the natural areas*”.



## 7 Reference:

- Abiven, S., Menasseri, S., Angers, D.A., Leterme, P., 2007. Dynamics of aggregate stability and biological binding agents during decomposition of organic materials. *European Journal of Soil Science* 58, 239–247. doi:10.1111/j.1365-2389.2006.00833.x
- Aciego Pietri, J.C., Brookes, P.C., 2009. Substrate inputs and pH as factors controlling microbial biomass, activity and community structure in an arable soil. *Soil Biology and Biochemistry* 41, 1396–1405. doi:10.1016/j.soilbio.2009.03.017
- Acton, Q.A. (Ed.), 2011. Microbiology, in: *Issues in Life Sciences: Muscle, Membrane and General Microbiology*. Acholarly Edition, Atlanta, Georgia, p. 1314.
- Agrifoods, F.F. and, 2017. News Releases [WWW Document]. Municipal Affairs.
- Agrios.N George, 2005. Plant Diseases Caused by Fungi, in: *Plant Pathology*. Academic Press INC, p. 292.
- Aislabie, J., Deslippe, J.R., Dymond, J.R., 2013. Soil microbes and their contribution to soil services. *Ecosystem Services in New Zealand - Conditions and Trends* 143–161.
- Altdorff, D., Galagedara, L., Unc, A., 2017. Impact of projected land conversion on water balance of boreal soils in western Newfoundland. *Journal of Water and Climate Change*.
- Arfi, Y., Marchand, C., Wartel, M., Record, E., 2012. Fungal diversity in anoxic-sulfidic sediments in a mangrove soil. *Fungal Ecology* 5, 282–285. doi:10.1016/j.funeco.2011.09.004
- Atkin, O.K., 1996. Reassessing the nitrogen relations of Arctic plants: a mini-review. *Plant, Cell and Environment* 19, 695–704. doi:10.1111/j.1365-3040.1996.tb00404.x
- Aziz, I., Ashraf, M., Mahmood, T., Islam, K., 2011. Crop rotation impact on soil quality. *Pak. J. Bot* 43, 949–960.
- Aziz, I., Mahmood, T., Islam, K.R., 2013. Soil & Tillage Research Effect of long term no-till and conventional tillage practices on soil quality. *Soil & Tillage Research* 131, 28–35. doi:10.1016/j.still.2013.03.002
- Baah-Acheamfour, M., Chang, S.X., Bork, E.W., Carlyle, C.N., 2017. The potential of agroforestry to reduce atmospheric greenhouse gases in Canada: Insight from pairwise comparisons with traditional agriculture, data gaps and future research. *Forestry Chronicle* 93, 180–189. doi:10.5558/tfc2017-024
- Bagchi, S., Roy, S., Maitra, A., Sran, R.S., 2017. Herbivores suppress soil microbes to influence carbon sequestration in the grazing ecosystem of the Trans-Himalaya. *Agriculture, Ecosystems and Environment* 239, 199–206. doi:10.1016/j.agee.2017.01.033
- Balmford, A., Bennun, L., Brink, B., Cooper, D., Côté, I.M., Crane, P., Dobson, A., Dudley, N., Dutton, I., Green, R.E., Gregory, R.D., Harrison, J., Kennedy, E.T., Kremen, C., Leader-williams, N., Lovejoy, T.E., Mace, G., May, R., Mayaux, P., Morling, P., Phillips, J., Redford, K., Ricketts, T.H., Rodríguez, J.P., Sanjayan, M., Schei, P.J., Jaarsveld, A.S. Van, Walther, B.A., 2010. The Convention on Biological Diversity ' s 2010 Target. *Science* 307, 212–213.
- Bandolin, T.H., Fisher, R.F., 1991. Agroforestry systems in North America. *Agroforestry Systems* 16, 95–118. doi:10.1007/BF00129742
- Bardgett, R., 2005. The Soil Environment, in: *The Biology of Soil*. Oxford University Press, pp. 1–23.
- Bargett, R.D., Yeates, G.W., Anderson, J.M., 2005. Patterns and Determination of Soil Biological Activity, in: *Biological Diversity and Function in Soils*. British Ecological Society, pp. 100–119.

- Barrios-González, J., Fernández, F.J., Tomasini, A., 2003. Microbial Secondary Metabolites Production and Strain Improvement. *Indian Journal of Biotechnology* 2, 322–333. doi:10.3389/fmicb.2014.00718
- Bartlová, J., Badalíková, B., Pospíšilová, L., Pokorný, E., Šarapatka, B., 2015. Water stability of soil aggregates in different systems of Tillage. *Soil and Water Research* 10, 147–154. doi:10.17221/132/2014-SWR
- Batten, J.W., Gibson, S.J., 1977. The Nature of Soils, in: *Soils: Their Nature, Classes, Distribution, Uses and Care*. The University of Alabama Press, Alabama, pp. 3–29.
- Bauer, R., Metzler, B., Begerow, D., Oberwinkler, F., 2009. *Cystobasidiopsis nirenbergiae*, a new agaricostilbomycete (Pucciniomycotina). *Mycological Research* 113, 960–966. doi:10.1016/j.mycres.2009.05.003
- Bell, T.H., El-Din Hassan, S., Lauron-Moreau, A., Al-Otaibi, F., Hijri, M., Yergeau, E., St-Arnaud, M., 2014. Linkage between bacterial and fungal rhizosphere communities in hydrocarbon-contaminated soils is related to plant phylogeny. *The ISME Journal* 8, 331–343. doi:10.1038/ismej.2013.149
- Bertrand, J.C., Caumette, P., Lebaron, P., Matheron, R., Normond, P., Sime-Ngando, T. (Eds.), 2011. The Major Phyla of Gram-Positive Bacteria: The Phylum Armatimonadetes, in: *Environmental Microbiology: Fundamentals and Applications*. Microbial Ecology. Springer Heidelberg New York Dordrecht London, p. 185.
- Bester, R., 2005. Growth and survival of *Saccharomyces cerevisiae* in soil.
- Bezdicsek, D.F., Beaver, T., Granatstein, D., 2003. Subsoil ridge tillage and lime effects on soil microbial activity, soil pH, erosion, and wheat and pea yield in the Pacific Northwest, USA. *Soil and Tillage Research* 74, 55–63. doi:10.1016/S0167-1987(03)00091-6
- Bossio, D.A., Girvan, M.S., Verchot, L., Bullimore, J., Borelli, T., Albrecht, A., Scow, K.M., Ball, A.S., Pretty, J.N., Osborn, A.M., 2005. Soil microbial community response to land use change in an agricultural landscape of western Kenya. *Microbial Ecology* 49, 50–62. doi:10.1007/s00248-003-0209-6
- Bossio, D.A., Scow, K.M., 1998. Impacts of carbon and flooding on soil microbial communities: Phospholipid fatty acid profiles and substrate utilization patterns. *Microbial Ecology* 35, 265–278. doi:10.1007/s002489900082
- Bridge, P., Spooner, B., 2001. Soil fungi diversity and detection.pdf. *Plant and Soil* 232, 147–154. doi:10.1023/A:1010346305799
- Bridges, E., 1978. Composition of Soils, in: *World Soils*. pp. 14–23.
- Brookes, P.C., Lauber, C.L., Rousk, J., Ba, E., Caporaso, J.G., Knight, R., Fierer, N., 2010. Soil bacterial and fungal communities across a pH gradient in an arable soil 1–12. doi:10.1038/ismej.2010.58
- Broughton, R.C.I., Newsham, K.K., Hill, P.W., Stott, A., Jones, D.L., 2015. Differential acquisition of amino acid and peptide enantiomers within the soil microbial community and its implications for carbon and nitrogen cycling in soil. *Soil Biology and Biochemistry* 88, 83–89. doi:10.1016/j.soilbio.2015.05.003
- Bryant, D.A., Liu, Z., 2013. Evolution of Green Bacteria, in: Thomas, B. (Ed.), *Advances in Botanical Research: Genome Evolution of Photosynthetic Bacteria*. Elsevier Ltd, pp. 125–126.
- Brzezińska, M., Nosalewicz, M., Pasztelan, M., Włodarczyk, T., 2012. Methane Production and Consumption in Loess Soil at Different Slope Position. *The Scientific World Journal* 2012, 1–8. doi:10.1100/2012/620270

- Button, R.G., 1983. Soils of Cormack-Deer Lake Area. Newfoundland.
- Canfora, L., Bacci, G., Pinzari, F., Lo Papa, G., Dazzi, C., Benedetti, A., 2014. Salinity and bacterial diversity: To what extent does the concentration of salt affect the bacterial community in a saline soil? PLoS ONE 9. doi:10.1371/journal.pone.0106662
- Cao, H., Chen, R., Wang, L., Jiang, L., Yang, F., Zheng, S., Wang, G., Lin, X., 2016. Soil pH, total phosphorus, climate and distance are the major factors influencing microbial activity at a regional spatial scale. Scientific Reports 6, 25815. doi:10.1038/srep25815
- Capriotti, A., 1955. Yeasts in some Netherlands soils. *Antonie van Leeuwenhoek* 21, 145–156. doi:10.1007/BF02543809
- Carlile, R., Coules, A., 2012. Control of Crop Diseases, Third Edit. ed. Cambridge University Press.
- Carol, S., Deborah, L., Joyce, L., 2004. Fungi in Fresh Water, in: Gregory, M.M., Gerald, B., Mercedes, F. (Eds.), *Biodiversity of Fungi Inventory and Monitoring Methods*. Elsevier Ltd, pp. 513–531.
- Chang, C., Sommerfeldt, T.G., Entz, T., 1990. APPLICATIONS OF CATTLE FEEDLOT MANURE. *Canadian Journal of Soil Science* 70, 673–681.
- Chapin, S.F., Matson, P.A., Mooney, H.A., 2002. Principles of Terrestrial Ecosystem Ecology. Vasa 1–436. doi:10.1007/978-1-4419-9504-9
- Chapin III, F.S., Moilanen, L., Kielland, K., 1993. Preferential use of organic nitrogen for growth by a non-mycorrhizal arctic sedge. *Nature* 361, 150–153. doi:10.1038/361150a0
- Chen, X.W., Liang, A.Z., Jia, S.X., Zhang, X.P., Wei, S.C., 2014. Impact of tillage on physical characteristics in a Mollisol of Northeast China 60, 309–313.
- Cheshire, M. V., 1979. Nature and Origin of Carbohydrates in Soils. Academic Press, London.
- Chesworth, W., 2006. Encyclopedia of soil science.
- Cho, S.-J., Kim, M.-H., Lee, Y.-O., 2016. Effect of pH on soil bacterial diversity. *Journal of Ecology and Environment* 40, 10. doi:10.1186/s41610-016-0004-1
- Clara, F., Dvm, T., Sc, M., n.d. Biochemistry of Enzymes, Carbohydrates & lipids.
- Colwell, R.K., Elsensohn, J.E., 2014. EstimateS turns 20: Statistical estimation of species richness and shared species from samples, with non-parametric extrapolation. *Ecography* 37, 609–613. doi:10.1111/ecog.00814
- Constancias, F., Saby, N.P.A., Terrat, S., Dequiedt, S., Horrigue, W., Nowak, V., Guillemin, J.P., Biju-Duval, L., Chemidlin Prévost-Bouré, N., Ranjard, L., 2015. Contrasting spatial patterns and ecological attributes of soil bacterial and archaeal taxa across a landscape. *MicrobiologyOpen* 4, 518–531. doi:10.1002/mbo3.256
- Cook, A.M., Hütter, R., 1981. s-Triazines as Nitrogen Sources for Bacteria. *Journal of Agricultural and Food Chemistry* 29, 1135–1143. doi:10.1021/jf00108a009
- Costanza, R., D'Arge, R., de Groot, R., Farber, S., Grasso, M., Hannon, B., Limburg, K., Naeem, S., O'Neill, R. V., Paruelo, J., Raskin, R.G., Sutton, P., van den Belt, M., 1998. The value of the world's ecosystem services and natural capital. *Nature* 387, 253–260. doi:10.1038/387253a0
- Crits-Christoph, A., Robinson, C.K., Barnum, T., Fricke, W.F., Davila, A.F., Jedynek, B., McKay, C.P., Diruggiero, J., 2013. Colonization patterns of soil microbial communities in the Atacama Desert. *Microbiome* 1, 28. doi:10.1186/2049-2618-1-28
- De Boer, W., Folman, L.B., Summerbell, R.C., Boddy, L., 2005. Living in a fungal world: Impact of fungi on soil bacterial niche development. *FEMS Microbiology Reviews* 29, 795–811. doi:10.1016/j.femsre.2004.11.005

- De Deyn, G.B., Cornelissen, J.H.C., Bardgett, R.D., 2008. Plant functional traits and soil carbon sequestration in contrasting biomes. *Ecology Letters* 11, 516–531. doi:10.1111/j.1461-0248.2008.01164.x
- de Menezes, A., Clipson, N., Doyle, E., 2012. Comparative metatranscriptomics reveals widespread community responses during phenanthrene degradation in soil. *Environmental Microbiology* 14, 2577–2588. doi:10.1111/j.1462-2920.2012.02781.x
- DeForest, J.L., Smemo, K.A., Burke, D.J., Elliott, H.L., Becker, J.C., 2012. Soil microbial responses to elevated phosphorus and pH in acidic temperate deciduous forests. *Biogeochemistry* 109, 189–202. doi:10.1007/s10533-011-9619-6
- Defries, R.S., Foley, J.A., Asner, G.P., 2004. Land-use choices : balancing human needs and ecosystem function In a nutshell :
- DeLuca, T.H., Zackrisson, O., Nilsson, M.-C., Sellstedt, A., 2002. Quantifying Nitrogen-Fixation in Feather Moss Carpets of Boreal Forests. *Letters to Nature* 419, 917–920. doi:10.1038/nature01136.1.
- Dequiedt, S., Saby, N.P.A., Lelievre, M., Jolivet, C., Thioulouse, J., Toutain, B., Arrouays, D., Bispo, A., Lemanceau, P., Ranjard, L., 2011. Biogeographical patterns of soil molecular microbial biomass as influenced by soil characteristics and management. *Global Ecology and Biogeography* 20, 641–652. doi:10.1111/j.1466-8238.2010.00628.x
- Dobrovol'skaya, T.G., Zvyagintsev, D.G., Chernov, I.Y., Golovchenko, A. V, Zenova, G.M., Lysak, L. V, Manucharova, N.A., Marfenina, O.E., Polyanskaya, L.M., Stepanov, A.L., Umarov, M.M., 2015. The role of microorganisms in the ecological functions of soils. *Eurasian Soil Science* 48, 959–967. doi:10.1134/s1064229315090033
- Douelle, A., 2014. Soil microaggregate and macroaggregate decay over time and soil carbon change as influenced by different tillage systems 69. doi:10.2489/jswc.69.6.574
- Dowd, R.W.O., Hopkins, D.W., 2009. Mineralization of Carbon From D - and L -Amino Acids and D -Glucose in Two Contrasting Soils. *Science* 30, 2009–2016.
- Egamberdieva, D., Kucharova, Z., 2008. Cropping effects on microbial population and nitrogenase activity in saline arid soil. *Turkish Journal of Biology* 32, 85–90.
- Eilers, K.G., Debenport, S., Anderson, S., Fierer, N., 2012. Digging deeper to find unique microbial communities: The strong effect of depth on the structure of bacterial and archaeal communities in soil. *Soil Biology and Biochemistry* 50, 58–65. doi:10.1016/j.soilbio.2012.03.011
- Eisenlord, S.D., Zak, D.R., Upchurch, R.A., 2012. Dispersal limitation and the assembly of soil Actinobacteria communities in a long-term chronosequence. *Ecology and Evolution* 2, 538–549. doi:10.1002/ece3.210
- Eldridge, D.J., Woodhouse, J.N., Curlevski, N.J. a, Hayward, M., Brown, M. V, Neilan, B. a, 2015. Soil-foraging animals alter the composition and co-occurrence of microbial communities in a desert shrubland. *The ISME Journal* 9, 1–11. doi:10.1038/ismej.2015.70
- EMBL-European Bioinformatics Institute (EMBL-EBI) [WWW Document], 1980. URL <https://www.ebi.ac.uk>
- Fahy, E., Cotter, D., Sud, M., Subramaniam, S., 2014. Lipid classification, structures and tools 1811, 637–647. doi:10.1016/j.bbalip.2011.06.009.Lipid
- Fai, P.B., Grant, A., 2009. A comparative study of *Saccharomyces cerevisiae* sensitivity against eight yeast species sensitivities to a range of toxicants. *Chemosphere* 75, 289–296. doi:10.1016/j.chemosphere.2008.12.059
- Fan, X., Yu, H., Wu, Q., Ma, J., Xu, H., Yang, J., Zhuang, Y., 2016. Effects of fertilization on

- microbial abundance and emissions of greenhouse gases (CH<sub>4</sub> and N<sub>2</sub>O) in rice paddy fields. *Ecology and Evolution* 6, 1054–1063. doi:10.1002/ece3.1879
- Fedotov, G.N., Lysak, L. V, 2014. The possible role of microorganisms in humus formation in soils. *Doklady Biological Sciences : Proceedings of the Academy of Sciences of the USSR, Biological Sciences Sections / Translated from Russian* 455, 87–90. doi:10.1134/S001249661402001X
- Fernández-Calviño, D., Bååth, E., 2010. Growth response of the bacterial community to pH in soils differing in pH. *FEMS Microbiology Ecology* 73, 149–156. doi:10.1111/j.1574-6941.2010.00873.x
- Fierer, N., Breitbart, M., Nulton, J., Salamon, P., Lozupone, C., Jones, R., Robeson, M., Edwards, R.A., Felts, B., Rayhawk, S., Knight, R., Rohwer, F., Jackson, R.B., 2007. Metagenomic and small-subunit rRNA analyses reveal the genetic diversity of bacteria, archaea, fungi, and viruses in soil. *Applied and Environmental Microbiology* 73, 7059–7066. doi:10.1128/AEM.00358-07
- Fierer, N., Jackson, R.B., 2006. The diversity and biogeography of soil bacterial communities. *Proceedings of the National Academy of Sciences of the United States of America* 103, 626–631. doi:10.1073/pnas.0507535103
- Fierer, N., Leff, J.W., Adams, B.J., Nielsen, U.N., Bates, S.T., Lauber, C.L., Owens, S., Gilbert, J.A., Wall, D.H., Caporaso, J.G., 2012. Cross-biome metagenomic analyses of soil microbial communities and their functional attributes. *Proceedings of the National Academy of Sciences* 109, 21390–21395. doi:10.1073/pnas.1215210110
- Fierer, N., Strickland, M.S., Liptzin, D., Bradford, M.A., Cleveland, C.C., 2009. Global patterns in belowground communities. *Ecology Letters* 12, 1238–1249. doi:10.1111/j.1461-0248.2009.01360.x
- Finn, D., Kopittke, P.M., Dennis, P.G., Dalal, R.C., 2017. Microbial energy and matter transformation in agricultural soils. *Soil Biology and Biochemistry* 111, 176–192. doi:10.1016/j.soilbio.2017.04.010
- Fortner, R. J., 2010. *From the Surface Down: An Introduction to Soil Surveys for Agronomic Use*. Agriculture.
- Francis, M.. (Ed.), 2014. *Advances in Botanical Research Fungi*, First Edit. ed. Elsevier Ltd.
- Frey, S.D., Lee, J., Melillo, J.M., Six, J., 2013. The temperature response of soil microbial efficiency and its feedback to climate. *Nature Climate Change* 3, 395–398. doi:10.1038/nclimate1796
- Fukunaga, Y., 2014. The Prokaryotes. doi:10.1007/978-3-642-38954-2
- Gaby, J.C., Buckley, D.H., 2014. A comprehensive aligned nifH gene database: A multipurpose tool for studies of nitrogen-fixing bacteria. *Database* 2014, 1–8. doi:10.1093/database/bau001
- Gahan, J., Schmalenberger, A., 2016. The Role of Bacteria and Mycorrhiza in Plant Sulfur Supply, in: *Frontiers of Sulfur Metabolism in Plant Growth, Development, and Stress Response*. pp. 22–26.
- Gans, J., Wolinsky, M., Dunbar, J., 2005. Computational Improvements Reveal Great Bacterial Diversity and High Metal Toxicity in Soil. *Science* 309, 1387–1390. doi:10.1126/science.1112665
- Garbeva, P., Van Veen, J.A., Van Elsas, J.D., 2003. Predominant *Bacillus* spp. in agricultural soil under different management regimes detected via PCR-DGGE. *Microbial Ecology* 45, 302–316. doi:10.1007/s00248-002-2034-8

- García-Orenes, F., Morugán-Coronado, A., Zornoza, R., Scow, K., 2013. Changes in Soil Microbial Community Structure Influenced by Agricultural Management Practices in a Mediterranean Agro-Ecosystem. *PLoS ONE* 8, e80522. doi:10.1371/journal.pone.0080522
- Gianfreda, Bollag, 1996. B. Classification of Functions, in: *Soil Biochemistry*, Volume 9. p. 130.
- Gioseffi, E., De Neergaard, A., Schjoerring, J.K., 2012. Interactions between uptake of amino acids and inorganic nitrogen in wheat plants. *Biogeosciences* 9, 1509–1518. doi:10.5194/bg-9-1509-2012
- Girvan, M.S., Bullimore, J., Pretty, J.N., Mark, a, Ball, A.S., Osborn, a M., 2003. Soil Type Is the Primary Determinant of the Composition of the Total and Active Bacterial Communities in Arable Soils Soil Type Is the Primary Determinant of the Composition of the Total and Active Bacterial Communities in Arable Soils. *Applied and Environmental Microbiology* 69, 1800–1809. doi:10.1128/AEM.69.3.1800
- Glazovsky, N.G. and, Nina, Z. (Eds.), 2009. Soil Biota, in: *Environmental Structure and Function: Earth System*. EOLSS, pp. 166–168.
- Gomes, N.C.M., Fagbola, O., Costa, R., Rumjanek, N.G., Buchner, A., Mendona-hagler, L., Smalla, K., 2003. Dynamics of Fungal Communities in Bulk and Maize Rhizosphere Soil in the Tropics Dynamics of Fungal Communities in Bulk and Maize Rhizosphere Soil in the Tropics. *Applied and Environmental Microbiology* 69, 3758–3766. doi:10.1128/AEM.69.7.3758
- Goodfellow, M., 2012. Phylum XXVI. Actinobacteria phyl. nov. *Bergey's Manual of Systematic Bacteriology* 3, 33–34. doi:10.1007/978-0-387-68233-4
- Gougoulas, C., Clark, J.M., Shaw, L.J., 2014. The role of soil microbes in the global carbon cycle: Tracking the below-ground microbial processing of plant-derived carbon for manipulating carbon dynamics in agricultural systems. *Journal of the Science of Food and Agriculture* 94, 2362–2371. doi:10.1002/jsfa.6577
- Govaerts, B., Mezzalama, M., Unno, Y., Sayre, K.D., Luna-guido, M., Vanherck, K., Dendooven, L., Deckers, J., 2007. Influence of tillage , residue management , and crop rotation on soil microbial biomass and catabolic diversity 37, 18–30. doi:10.1016/j.apsoil.2007.03.006
- Green Genes [WWW Document], 2005. URL <http://greengenes.lbl.gov/Download/>
- Gregorich, E., Carter, M.R., Doran, J., Pankhurst, C., Dwyer, L., 1997. Biological Attributes of Soil Quality, in: *Soil Quality for Crop Production and Ecosystem Health*. Elsevier, pp. 81–112.
- Griffiths, B.S., Philippot, L., 2013. Insights into the resistance and resilience of the soil microbial community. *FEMS Microbiology Reviews* 37, 112–129. doi:10.1111/j.1574-6976.2012.00343.x
- Grissa, I., Bidard, F., Grognet, P., Grossetete, S., Silar, P., 2010. The Nox/Ferric reductase/Ferric reductase-like families of Eumycetes. *Fungal Biology* 114, 766–777. doi:10.1016/j.funbio.2010.07.002
- Grote, J., Labrenz, M., Pfeiffer, B., Jost, G., Jürgens, K., 2007. Quantitative distributions of Epsilonproteobactena and a Sulfurimonas subgroup in pelagic redoxclines of the central Baltic Sea. *Applied and Environmental Microbiology* 73, 7155–7161. doi:10.1128/AEM.00466-07
- Grünzweig, J.M., Sparrow, S.D., Chapin III, S.F., 2003. Impact of Forest Conversion to Agriculture on Carbon and Nitrogen Mineralization in Subarctic Alaska 64, 271–296.
- Gryndler, M., Larsen, J., Hrselova, H., Rezacova, V., Gryndleroca, H., Kubat, J., 2006. Organic

- and mineral fertilization , respectively , increase and decrease the development of external mycelium of arbuscular mycorrhizal fungi in a long-term field experiment 159–166. doi:10.1007/s00572-005-0027-4
- Gueidan, C., David, H., Miaklikowska, J., Lutzoni, F., 2014. Pezizomycotina: Lecanoromycetes, in: Esser, K. (Ed.), *The Mycota: A Comprehensive Treatise on Fungi as Experimental System for Basic and Applied Research (Systematics and Evolution Part A)*. Springer Heidelberg New York Dordrecht London, pp. 89–108.
- Gundale, M.J., Deluca, T.H., Nordin, A., 2011. Bryophytes attenuate anthropogenic nitrogen inputs in boreal forests. *Global Change Biology* 17, 2743–2753. doi:10.1111/j.1365-2486.2011.02407.x
- Gundale, M.J., Wardle, D.A., Nilsson, M., Ecology, S., June, N., Gundale, J., 2015. Vascular plant removal effects on biological N fixation vary across a boreal forest island gradient Published by : Ecological Society of America Linked references are available on JSTOR for this article : Your use of the JSTOR archive indicates your accep 91, 1704–1714.
- Haichar, F. el Z., Marol, C., Berge, O., Rangel-Castro, J.I., Prosser, J.I., Balesdent, J., Heulin, T., Achouak, W., 2008. Plant host habitat and root exudates shape soil bacterial community structure. *The ISME Journal* 2, 1221–1230. doi:10.1038/ismej.2008.80
- Halling, E.R.E., 2017. Ectomycorrhizae : Co-Evolution , Significance , and Biogeography Author ( s ) : Roy E . Halling Source : *Annals of the Missouri Botanical Garden* , Vol . 88 , No . 1 ( Winter , 2001 ), pp . 5-13 Published by : Missouri Botanical Garden Press Stable URL : ht 88, 5–13.
- Hammer, Ø., 2017. PAST Paleontological Statistics Version 3.17 151–152.
- Harwood, L.J., 1984. *Lipids in Plants and Microbes*. Springer Netherlands.
- Hayat, R., Ali, S., Amara, U., Khalid, R., Ahmad, I., 2010. Soil Beneficial Bacteria and their Role in Plant Growth Promotion: a Review. *Annals of Microbiology* 60, 579–598. doi:https://doi.org/10.1007/s13213-010-0117-1
- He, R., Yang, K., Li, Z., Schädler, M., Yang, W., Wu, F., Tan, B., Zhang, L., Xu, Z., 2017. Effects of forest conversion on soil microbial communities depend on soil layer on the eastern Tibetan Plateau of China. *Plos One* 12, e0186053. doi:10.1371/journal.pone.0186053
- He, S., Guo, L., Niu, M., Miao, F., Jiao, S., Hu, T., Long, M., 2017. Ecological diversity and co-occurrence patterns of bacterial community through soil profile in response to long-term switchgrass cultivation. *Scientific Reports* 7, 3608. doi:10.1038/s41598-017-03778-7
- Hibbett, D.S., Binder, M., Bischoff, J.F., Blackwell, M., Cannon, P.F., Eriksson, O.E., Huhndorf, S., James, T., Kirk, P.M., Lücking, R., Thorsten Lumbsch, H., Lutzoni, F., Matheny, P.B., McLaughlin, D.J., Powell, M.J., Redhead, S., Schoch, C.L., Spatafora, J.W., Stalpers, J.A., Vilgalys, R., Aime, M.C., Aptroot, A., Bauer, R., Begerow, D., Benny, G.L., Castlebury, L.A., Crous, P.W., Dai, Y.C., Gams, W., Geiser, D.M., Griffith, G.W., Gueidan, C., Hawksworth, D.L., Hestmark, G., Hosaka, K., Humber, R.A., Hyde, K.D., Ironside, J.E., Kõljalg, U., Kurtzman, C.P., Larsson, K.H., Lichtwardt, R., Longcore, J., Miadlikowska, J., Miller, A., Moncalvo, J.M., Mozley-Standridge, S., Oberwinkler, F., Parmasto, E., Reeb, V., Rogers, J.D., Roux, C., Ryvarden, L., Sampaio, J.P., Schüßler, A., Sugiyama, J., Thorn, R.G., Tibell, L., Untereiner, W.A., Walker, C., Wang, Z., Weir, A., Weiss, M., White, M.M., Winka, K., Yao, Y.J., Zhang, N., 2007. A higher-level phylogenetic classification of the Fungi. *Mycological Research* 111, 509–547. doi:10.1016/j.mycres.2007.03.004
- Hill, P.W., Farrell, M., Roberts, P., Farrar, J., Grant, H., Newsham, K.K., Hopkins, D.W.,

- Bardgett, R.D., Jones, D.L., 2011a. Soil- and enantiomer-specific metabolism of amino acids and their peptides by Antarctic soil microorganisms. *Soil Biology and Biochemistry* 43, 2410–2416. doi:10.1016/j.soilbio.2011.08.006
- Hill, P.W., Quilliam, R.S., DeLuca, T.H., Farrar, J., Farrell, M., Roberts, P., Newsham, K.K., Hopkins, D.W., Bardgett, R.D., Jones, D.L., 2011b. Acquisition and assimilation of nitrogen as peptide-bound and D-enantiomers of amino acids by wheat. *PLoS ONE* 6, 6–9. doi:10.1371/journal.pone.0019220
- Hobara, S., Osono, T., Hirose, D., Noro, K., Hirota, M., Benner, R., 2014. The roles of microorganisms in litter decomposition and soil formation. *Biogeochemistry* 118, 471–486. doi:10.1007/s10533-013-9912-7
- Hobbs, P.R., Sayre, K., Gupta, R., 2008. The role of conservation agriculture in sustainable agriculture. *Philosophical Transactions of the Royal Society B: Biological Sciences* 363, 543–555. doi:10.1098/rstb.2007.2169
- Hofstra, N., Bouwman, A.F., 2005. Denitrification in agricultural soils: Summarizing published data and estimating global annual rates. *Nutrient Cycling in Agroecosystems* 72, 267–278. doi:10.1007/s10705-005-3109-y
- Hokayem, B., Azzi, D. El, 2014. Fate of Copper and Zinc in Cattle Manure. *International Conference on Chemical, Environment & Biological Sciences* 183–188. doi:10.15242/IICBE.C914117
- Holland, A.E., Coleman, C.D., 2015. Litter Placement Effects on Microbial and Organic Matter Dynamics in an Agroecosystem Author (s): E. A. Holland and D. C. Coleman Published by: Ecological Society of America LITTER PLACEMENT EFFECTS ON MICROBIAL AND ORGANIC MATTER DYNAMICS IN AN AG. *Ecology Society of America* 68, 425–433.
- Hoorman, J.J., 2011. The Role of Soil Fungus. *Agriculture and Natural Resources* 1–6.
- Hopkins, D.W., O'Dowd, R.W., Shiel, R.S., 1997. Comparison of D- and L-Amino Acid Metabolism in Soils With Differing Microbial Biomass and Activity 29, 23–29.
- Horn, M.A., Ihsen, J., Matthies, C., Wüst, P.K., Depkat-jakob, P., Harold, L., n.d. Microbial Ecology of Flavobacteria in Soil-Potential Implication for Greenhouse Gas Production: Flavobacterium denitrificans as Transient inhabitant of the Earthworm Gut 6–8.
- Houghton, R.A., Hackler, J.L., Lawrence, K.T., 1999. The U. S. Carbon Budget: Contributions from Land-Use Change 285, 574–579.
- Howard, H.P., 1989. Hand book of Environmental Fate and Exposure Data for Organic Chemicals.
- Huson, D., Auch, A., Qi, J., Schuster, S., 2007. MEGAN analysis of metagenome data. *Genome Res.* 17, 377–386. doi:10.1101/gr.5969107.
- Ishii, S., Ikeda, S., Minamisawa, K., Senoo, K., 2011. Nitrogen Cycling in Rice Paddy Environments: Past Achievements and Future Challenges. *Microbes and Environments* 26, 282–292. doi:10.1264/jsme2.ME11293
- Islam, K.R., Weil, R.R., 2000. Land use effects on soil quality in a tropical forest ecosystem of Bangladesh. *Agriculture Ecosystems & Environment* 79, 9–16. doi:10.1016/S0167-8809(99)00145-0
- Jämtgård, S., 2010. The Occurrence of Amino Acids in Agricultural Soil and their Uptake by Plants.
- Jasrotia, P., Green, S.J., Canion, A., Overholt, W.A., Prakash, O., Wafula, D., Hubbard, D., Watson, D.B., Schadt, C.W., Brooks, S.C., Kostka, J.E., 2014. Watershed-scale fungal



- community characterization along a pH gradient in a subsurface environment cocontaminated with uranium and nitrate. *Applied and Environmental Microbiology* 80, 1810–1820. doi:10.1128/AEM.03423-13
- Jassey, V.E., Chiapusio, G., Binet, P., Buttler, A., Laggoun-Défarge, F., Delarue, F., Bernard, N., Mitchell, E.A., Toussaint, M.L., Francez, A.J., Gilbert, D., 2013. Above- and belowground linkages in Sphagnum peatland: Climate warming affects plant-microbial interactions. *Global Change Biology* 19, 811–823. doi:10.1111/gcb.12075
- Jeffery, S., Harris, J.A., Rickson, R.J., Ritz, K., 2009. The spectral quality of light influences the temporal development of the microbial phenotype at the arable soil surface. *Soil Biology and Biochemistry* 41, 553–560. doi:10.1016/j.soilbio.2008.12.014
- Joann, K.W., Luis, S., 2009. Soil Organisms and the Soil Foodweb, in: *Soil Ecology and Management*. pp. 29–63.
- Jobbágy, E.G., Jackson, R.B., 2000. The vertical distribution of soil organic carbon and its relation to climate and vegetation. *Ecological Applications* 10, 423–436. doi:10.1890/1051-0761(2000)010[0423:TVDOSO]2.0.CO;2
- Joergensen, R.G., Wichern, F., 2008. Quantitative assessment of the fungal contribution to microbial tissue in soil. *Soil Biology and Biochemistry* 40, 2977–2991. doi:10.1016/j.soilbio.2008.08.017
- Johnson, E. C., Johnson, H. A., Huntington, G. T., Siccama, G. T., 1989. Whole-Tree Clear-Cutting Effects on Soil Horizons and Organic Matter Pools. *Soil Science Society of America Journal* 55, 497–502.
- Johnson, R.D., 2011. *The Nitrogen Cycle*.
- Jolley, K., 2010. Bacterial Isolate Genome Sequence Database (BIGSdb) [WWW Document]. University of Oxford. URL <https://pubmlst.org/software/database/bigsdb/>
- Jones, C.M., Stres, B., Rosenquist, M., Hallin, S., 2008. Phylogenetic analysis of nitrite, nitric oxide, and nitrous oxide respiratory enzymes reveal a complex evolutionary history for denitrification. *Molecular Biology and Evolution* 25, 1955–1966. doi:10.1093/molbev/msn146
- Jones, G., 1970. *Studies on Lipids of Soil Micro-organisms with* 59.
- Jordan, D., Ponder, F., Hubbard, V.C., 2003. Effects of soil compaction, forest leaf litter and nitrogen fertilizer on two oak species and microbial activity. *Applied Soil Ecology* 23, 33–41. doi:10.1016/S0929-1393(03)00003-9
- Jumas-bilak, E., Pathoge, E., Re, C.H., Re, C.H., Bacte, L. De, 2014. *The Prokaryotes*. doi:10.1007/978-3-642-38954-2
- Kaiser, C., Koranda, M., Kitzler, B., Fuchslueger, L., Schnecker, J., Schweiger, P., Rasche, F., Zechmeister-Boltenstern, S., Sessitsch, A., Richter, A., 2010. Belowground carbon allocation by trees drives seasonal patterns of extracellular enzyme activities by altering microbial community composition in a beech forest soil. *New Phytologist* 187, 843–858. doi:10.1111/j.1469-8137.2010.03321.x
- Karhu, K., Auffret, M.D., Dungait, J.A.J., Hopkins, D.W., Prosser, J.I., Singh, B.K., Subke, J.A., Wookey, P.A., Agren, G.I., Sebastià, M.T., Gouriveau, F., Bergkvist, G., Meir, P., Nottingham, A.T., Salinas, N., Hartley, I.P., 2014. Temperature sensitivity of soil respiration rates enhanced by microbial community response. *Nature* 513, 81–84. doi:10.1038/nature13604
- Karlovsky, P., 2008. Secondary Metabolites in Soil Ecology 14, 1–3. doi:10.1007/978-3-540-74543-3

- Karpov, S.A., Mamanazarova, K.S., Popova, O. V., Aleoshin, V. V., James, T.Y., Mamkaeva, M.A., Tsvetkova, V.S., Vishnyakov, A.E., Longcore, J.E., 2017. Monoblepharidomycetes diversity includes new parasitic and saprotrophic species with highly intronized rDNA. *Fungal Biology* 121, 729–741. doi:10.1016/j.funbio.2017.05.002
- Khan, A.A., Jilani, G., Akhtar, M.S., Saqlan, S.M., Rasheed, M., 2009. Phosphorus Solubilizing Bacteria: Occurrence, Mechanisms and their Role in Crop Production. *Journal of Agriculture and Biological Sciences* 1, 48–58. doi:10.5923/j.re.20120201.10
- Kibblewhite, M., Ritz, K., Swift, M., 2008. Soil health in agricultural systems. *Philosophical Transactions of the Royal Society B: Biological Sciences* 363, 685–701. doi:10.1098/rstb.2007.2178
- Kim, M., Cho, A., Lim, H.S., Hong, S.G., Kim, J.H., Lee, J., Choi, T., Ahn, T.S., Kim, O.S., 2015. Highly heterogeneous soil bacterial communities around terra nova bay of Northern Victoria Land, Antarctica. *PLoS ONE* 10, 1–14. doi:10.1371/journal.pone.0119966
- King, A.J., Freeman, K.R., McCormick, K.F., Lynch, R.C., Lozupone, C., Knight, R., Schmidt, S.K., 2010. Biogeography and habitat modelling of high-alpine bacteria. *Nature Communications* 1, 1–6. doi:10.1038/ncomms1055
- Klimek, B., Chodak, M., Jaźwa, M., Niklińska, M., 2016. Functional diversity of soil microbial communities in boreal and temperate Scots pine forests. *European Journal of Forest Research* 135, 731–742. doi:10.1007/s10342-016-0968-5
- Knight, S.J., Goddard, M.R., 2015. Sporulation in soil as an overwinter survival strategy in *Saccharomyces cerevisiae*. *FEMS Yeast Research* 16, 1–8. doi:10.1093/femsyr/fov102
- Konstan, U., 1981. The bacteria of the sulphur cycle 441, 433–441.
- Kovács, P., Van Scoyoc, G.E., Doerge, T.A., Camberato, J.J., Vyn, T.J., 2014. Pre-plant anhydrous ammonia placement consequences on no-till versus conventional-till maize growth and nitrogen responses. *Agronomy Journal* 106, 634–644. doi:10.2134/agronj2013.0356
- Krashevskaya, V., Klärner, B., Widyastuti, R., Maraun, M., Scheu, S., 2015. Impact of tropical lowland rainforest conversion into rubber and oil palm plantations on soil microbial communities. *Biology and Fertility of Soils* 51, 697–705. doi:10.1007/s00374-015-1021-4
- Lagerström, A., Nilsson, M.C., Zackrisson, O., Wardle, D.A., 2007. Ecosystem input of nitrogen through biological fixation in feather mosses during ecosystem retrogression. *Functional Ecology* 21, 1027–1033. doi:10.1111/j.1365-2435.2007.01331.x
- Laliberté, E., Declerck, F., Metcalfe, J., Catterall, C.P., Sa, D., 2010. Land-use intensification reduces functional redundancy and response diversity in plant communities 76–86. doi:10.1111/j.1461-0248.2009.01403.x
- Landeweert, R., Hoffland, E., Finlay, R.D., Kuyper, T.W., Van Breemen, N., 2001. Linking plants to rocks: Ectomycorrhizal fungi mobilize nutrients from minerals. *Trends in Ecology and Evolution* 16, 248–254. doi:10.1016/S0169-5347(01)02122-X
- Langille, M.G.I., Zaneveld, J., Caporaso, J.G., McDonald, D., Knights, D., Reyes, J.A., Clemente, J.C., Burkepille, D.F., Vega Thurber, R.L., Knight, R., Beiko, R.G., Huttenhower, C., 2013. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. *Nature Biotechnology* 31, 1814–821. doi:10.1038/nbt.2676
- Lauber, C.L., Hamady, M., Knight, R., Fierer, N., 2009. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. *Applied and Environmental Microbiology* 75, 5111–5120. doi:10.1128/AEM.00335-09
- Lauber, C.L., Ramirez, K.S., Aanderud, Z., Lennon, J., Fierer, N., 2013. Temporal variability in

- soil microbial communities across land-use types. *The ISME Journal* 7, 1641–1650. doi:10.1038/ismej.2013.50
- Lauber, C.L., Strickland, M.S., Bradford, M.A., Fierer, N., 2008. The influence of soil properties on the structure of bacterial and fungal communities across land-use types. *Soil Biology and Biochemistry* 40, 2407–2415. doi:10.1016/j.soilbio.2008.05.021
- Lawton, K., Kurtz, L., 1957. Soil Reaction and Liming, in: *Soil*. pp. 184–193.
- Lazcano, C., Gómez-brandón, M., Revilla, P., Domínguez, J., 2012. Short-term effects of organic and inorganic fertilizers on soil microbial community structure and function. doi:10.1007/s00374-012-0761-7
- Le Mer, J., Roger, P., 2001. Production, oxidation, emission and consumption of methane by soils: A review. *European Journal of Soil Biology* 37, 25–50. doi:10.1016/S1164-5563(01)01067-6
- Lee, J.J., Lee, H.J., Jang, G.S., Yu, J.M., Cha, J.Y., Kim, S.J., Lee, E.B., Kim, M.K., 2013. *Deinococcus swuensis* sp. nov., a gamma-radiation-resistant bacterium isolated from soil. *Journal of Microbiology* 51, 305–311. doi:10.1007/s12275-013-3023-y
- Lee, Y.H., Kim, M.K., Lee, J., Heo, J.Y., Kang, T.H., Kim, H., Yun, H.D., 2013. Organic fertilizer application increases biomass and proportion of fungi in the soil microbial community in a minimum tillage Chinese cabbage field. *Canadian Journal of Soil Science* 93, 271–278. doi:10.4141/cjss2012-101
- Lepleux, C., Turpault, M.P., Oger, P., Frey-Klett, P., Uroz, S., 2012. Correlation of the abundance of betaproteobacteria on mineral surfaces with mineral weathering in forest soils. *Applied and Environmental Microbiology* 78, 7114–7119. doi:10.1128/AEM.00996-12
- Li, Y., Chapman, S.J., Nicol, G.W., Yao, H., 2018. Nitrification and nitrifiers in acidic soils. *Soil Biology and Biochemistry* 116, 290–301. doi:https://doi.org/10.1016/j.soilbio.2017.10.023
- Ling, Y.C., Bush, R., Grice, K., Tulipani, S., Berwick, L., Moreau, J.W., 2015. Distribution of iron- and sulfate-reducing bacteria across a coastal acid sulfate soil (CASS) environment: Implications for passive bioremediation by tidal inundation. *Frontiers in Microbiology* 6. doi:10.3389/fmicb.2015.00624
- Linn, D.M., Doran, J.W., 1984. Aerobic and Anaerobic Microbial Populations in No-till and Plowed Soils. *Soil Science Society of America Journal* 48, 794. doi:10.2136/sssaj1984.03615995004800040019x
- List of Toxic Substances Managed Under CEPA (Schedule 1)- Dichlorodiphenyltrichloroethane [WWW Document], 2013. . Environment and Climate Change Canada.
- Liu, X.Z., Wang, Q.M., Göker, M., Groenewald, M., Kachalkin, A. V., Lumbsch, H.T., Millanes, A.M., Wedin, M., Yurkov, A.M., Boekhout, T., Bai, F.Y., 2015. Towards an integrated phylogenetic classification of the Tremellomycetes. *Studies in Mycology* 81, 85–147. doi:10.1016/j.simyco.2015.12.001
- Lu, M., Zhang, Z.Z., 2014. Phytoremediation of soil co-contaminated with heavy metals and deca-BDE by co-planting of *Sedum alfredii* with tall fescue associated with *Bacillus cereus* JP12. *Plant and Soil* 382, 89–102. doi:10.1007/s11104-014-2147-0
- Lundgren, B.O., Raintree, J.B., 1983. Sustained Agroforestry. Reprint from *Agricultural Research for Development: Potentials and Challenges in Asia* 1983, 1–26.
- Luo, P., Han, X., Wang, Y., Han, M., Shi, H., Liu, N., Bai, H., 2015. Influence of long-term fertilization on soil microbial biomass, dehydrogenase activity, and bacterial and fungal community structure in a brown soil of northeast China. *Annals of Microbiology* 65, 533–542. doi:10.1007/s13213-014-0889-9

- Lupwayi, N., Rice, W., Clayton, G., 1998. Soil microbial diversity and community structure under wheat as influenced by tillage and crop rotation. *Soil Biology and Biochemistry* 30, 1733–1741. doi:10.1016/S0038-0717(98)00025-X
- M. Catherine Atme, Merje Toome, D.J.M., 2014. Pucciniomycotina, in: Esser, K. (Ed.), *The Mycota: A Comprehensive Treatise on Fungi as Experimental System for Basic and Applied Research (Systematics and Evolution Part A)*. Springer Heidelberg New York Dordrecht Londen, pp. 278, 286–287.
- Mackey, E. a, Lindstrom, R.M., Murphy, K.E., 2010. Certification of Three NIST Renewal Soil Standard Reference Materials for Element Content : SRM 2709a San Joaquin Soil , SRM 2710a Montana Soil I , and SRM 2711a Montana Soil II NIST Special Publication 260-172 Certification of Three NIST Renewal Soil Sta. National Institute of Standards and Technology Special Publication 260–172.
- Mader, P., Fliebbach, A., Dubois, D., Gunst, L., Fried, P., Niggli, U., 2002. Soil Fertility and Biodiversity in Organic Farming. *Science* 296, 1694–1697. doi:10.1126/science.1071148
- Maire, V., Gross, N., Börger, L., Proulx, R., Wirth, C., Pontes, L. da S., Soussana, J.F., Louault, F., 2012. Habitat filtering and niche differentiation jointly explain species relative abundance within grassland communities along fertility and disturbance gradients. *New Phytologist* 196, 497–509. doi:10.1111/j.1469-8137.2012.04287.x
- Mandal, U.K., 1998. Soil Physical and Chemical Properties in Relation to Conservation of natural Resource i.
- Marschner, P., Rengel, Z., 2007. Soil Biology- Nutrient Cycling in Terrestrial Ecosystems, *Soil Biology*. doi:10.1017/CBO9781107415324.004
- Martin, F., Torelli, S., Le Paslier, D., Barbance, A., Martin-Laurent, F., Bru, D., Geremia, R., Blake, G., Jouanneau, Y., 2012. Betaproteobacteria dominance and diversity shifts in the bacterial community of a PAH-contaminated soil exposed to phenanthrene. *Environmental Pollution* 162, 345–353. doi:10.1016/j.envpol.2011.11.032
- Marusenko, Y., Bates, S.T., Anderson, I., Johnson, S.L., Soule, T., Garcia-Pichel, F., 2013. Ammonia-oxidizing archaea and bacteria are structured by geography in biological soil crusts across North American arid lands. *Ecological Processes* 2, 9. doi:10.1186/2192-1709-2-9
- Mathew, R.P., Feng, Y., Githinji, L., Ankumah, R., Balkcom, K.S., 2012. Impact of No-Tillage and Conventional Tillage Systems on Soil Microbial Communities 2012. doi:10.1155/2012/548620
- Matson, P.A., Parton, W.J., Power, A.G., Swift, M.J., 1997. Agricultural Intensification and Ecosystem Properties 277.
- Mazzetto, A.M., Feigl, B.J., Cerri, C.E.P., Cerri, C.C., 2016. Comparing how land use change impacts soil microbial catabolic respiration in Southwestern Amazon. *Brazilian Journal of Microbiology* 47, 63–72. doi:10.1016/j.bjm.2015.11.025
- McCaughy, J., 1986. Energy Balance Measurment System for Agroforestry Application in Newfoundland.
- Mccauley, A., Jones, C., Olson-Rutz, K., 2017. Soil pH and Organic Matter. *Montana State University* 1–16.
- McGill, W.B., Cannon, K.R., Robertson, J.A., Cook, F.D., 1986. Organic C in Breton L After 50 Years of Cropping To Two. *Canadian Jouranl of Soil Science* 66, 1–19.
- McLaughlin, D.J., Atme, C.M., Toome, M., 2014. Pucciniomycotina, in: Esser, K. (Ed.), *The Mycota: A Comprehensive Treatise on Fungi as Experimental System for Basic and Applied*

- Research (Systematics and Evolution Part A). Springer Heidelberg New York Dordrecht London, p. 278, 286–287.
- Micheal Wess Jose Paulo Sampaio Robbert Bauer, F.O., 2014. Tremellomycetes and Related Groups, in: Esser, K. (Ed.), *The Mycota: A Comprehensive Treatise on Fungi as Experimental Systems for Basic and Applied Research (Systematics and Evolution Part 1)*. Springer Heidelberg New York Dordrecht London, pp. 331–352.
- Milosevic, N., Govedarica, M., 2002. Effect of herbicides on microbiological properties of soil. *Zbornik Matice Srpske Za Prirodne Nauke* 5–21. doi:10.2298/ZMSPN0201005M
- Mining, O.D., 2016. Orange Visual Programming Documentation.
- Mirza, B.S., Potisap, C., Nüsslein, K., Bohannan, B.J.M., Rodriguesa, J.L.M., 2014. Response of free-living nitrogen-fixing microorganisms to land use change in the amazon rainforest. *Applied and Environmental Microbiology* 80, 281–288. doi:10.1128/AEM.02362-13
- Miskovic, K., Rasovic, B., Sobieszczanski, J., Brankov, L., 1983. *Effect of Different Doses of U46 on Weed.pdf*.
- Moe, L.A., 2013. Amino acids in the rhizosphere: From plants to microbes. *American Journal of Botany* 100, 1692–1705. doi:10.3732/ajb.1300033
- Mohammadi-Sichani, M.M., Assadi, M.M., Farazmand, A., Kianirad, M., Ahadi, A.M., Ghahderijani, H.H., 2017. Bioremediation of soil contaminated crude oil by *Agaricomycetes*. *Journal of Environmental Health Science and Engineering* 15, 8. doi:10.1186/s40201-016-0263-x
- Mohanty, S.R., Bodelier, P.L.E., Floris, V., Mohanty, S.R., Bodelier, P.L.E., Floris, V., Conrad, R., 2006. Differential Effects of Nitrogenous Fertilizers on Methane-Consuming Microbes in Rice Field and Forest Soils Differential Effects of Nitrogenous Fertilizers on Methane-Consuming Microbes in Rice Field and Forest Soils †‡. *Applied and Environmental Microbiology* 72, 1346–1354. doi:10.1128/AEM.72.2.1346
- Mokma, D.L., Buurman, P., 1987. Podzols and Podzolization in Temperate Regions, *Soil Science*. doi:10.1097/00010694-198707000-00014
- Mueller, R.C., Belnap, J., Kuske, C.R., 2016. Soil Bacterial and Fungal Community Responses to Nitrogen Addition Across Soil Depth and Microhabitat in an Arid Shrubland, in: Makhallanyane, P., T., Cowan, D., Ramond, J.-B. (Eds.), *Microbial Ecology of Arid Terrestrial Systems*. *Frontiers in Microbiology*, p. 72.
- Murayama, S., 1984. Changes in the monosaccharide composition during the decomposition of straws under field conditions. *Soil Science and Plant Nutrition* 30, 367–381. doi:10.1080/00380768.1984.10434702
- Muturi, E.J., Donthu, R.K., Fields, C.J., Moise, I.K., Kim, C., 2017. Effect of pesticides on microbial communities in container aquatic habitats. *Nature Publishing Group* 1–10. doi:10.1038/srep44565
- Nacke, H., Goldmann, K., Schöning, I., Pfeiffer, B., Kaiser, K., Villamizar, G.A.C., Schrumpf, M., Buscot, F., Daniel, R., Wubet, T., 2016. Fine spatial scale variation of soil microbial communities under European beech and Norway spruce. *Frontiers in Microbiology* 7. doi:10.3389/fmicb.2016.02067
- Nacke, H., Thürmer, A., Wollherr, A., Will, C., Hodac, L., Herold, N., Schöning, I., Schrumpf, M., Daniel, R., 2011. Pyrosequencing-based assessment of bacterial community structure along different management types in German forest and grassland soils. *PLoS ONE* 6. doi:10.1371/journal.pone.0017000
- Nagy, L.G., Riley, R., Tritt, A., Adam, C., Daum, C., Floudas, D., Sun, H., Yadav, J.S.,

- Pangilinan, J., Larsson, K.H., Matsuura, K., Barry, K., Labutti, K., Kuo, R., Ohm, R.A., Bhattacharya, S.S., Shirouzu, T., Yoshinaga, Y., Martin, F.M., Grigoriev, I. V., Hibbett, D.S., 2016. Comparative genomics of early-diverging mushroom-forming fungi provides insights into the origins of lignocellulose decay capabilities. *Molecular Biology and Evolution* 33, 959–970. doi:10.1093/molbev/msv337
- Nasholm, T., Kielland, K., Ganeteg, U., 2008. Uptake of Organic Nitrogen by Plants. *New Phytologist* 243–255. doi:10.1111/j.1469-8137.2004.01263.x
- Nath, R., Samanta, R., 2012. Soil pH , microbial population , nitrate reductase and alkaline phosphatase activities of different environment of Dibrugarh district , Assam. *Pelagia Research Library* 3, 1772–1775.
- Naveed, M., Herath, L., Moldrup, P., Arthur, E., Nicolaisen, M., Norgaard, T., Ferré, T.P.A., de Jonge, L.W., 2016. Spatial variability of microbial richness and diversity and relationships with soil organic carbon, texture and structure across an agricultural field. *Applied Soil Ecology* 103, 44–55. doi:10.1016/j.apsoil.2016.03.004
- Nielsen, U.N., Wall, D.H., Six, J., 2015. Soil Biodiversity and the Environment. *Annual Review of Environment & Resources* 40, 63–90. doi:10.1146/annurev-environ-102014-021257
- Nikel, I., P., 2016. Genomics, in: *Microbial Models: From Environmental to Industrial Sustainability*. pp. 11–12.
- Nivelle, E., Verzeaux, J., Habbib, H., Kuzyakov, Y., Decocq, G., Roger, D., Lacoux, J., Duclercq, J., Spicher, F., Nava-saucedo, J., Catterou, M., Dubois, F., Tetu, T., 2016. Functional response of soil microbial communities to tillage , cover crops and nitrogen fertilization. *Applied Soil Ecology* 108, 147–155. doi:10.1016/j.apsoil.2016.08.004
- Nunan, N., Wu, K., Young, I.M., Crawford, J.W., Ritz, K., 2003. Spatial distribution of bacterial communities and their relationships with the micro-architecture of soil. *FEMS Microbiology Ecology* 44, 203–215. doi:10.1016/S0168-6496(03)00027-8
- Nunan, N., Wu, K., Young, I.M., Crawford, J.W., Ritz, K., 2002. In situ spatial patterns of soil bacterial populations, mapped at multiple scales, in an arable soil. *Microbial Ecology* 44, 296–305. doi:10.1007/s00248-002-2021-0
- O'Donnell, A.G., Goodfellow, M., Hawksworth, D.L., 1994. Theoretical and practical aspects of the quantification of biodiversity among microorganisms. *Philos Trans R Soc Lond B Biol Sci* 345, 65–73. doi:10.1098/rstb.1994.0087
- Oloo, K.P., Whalen, J.K., Chang, C., Bakry, M. a a, Soliman, Y.R. a, Moussa, S. a M., Eghball, B., Wienhold, B.J., Gilley, J.E., Eigenberg, R.A., Santillan, M. Y., Moreno, F. P., Garcia, F. P., Sandoval, A.A.O., Brown, C., Chang, C., Sommerfeldt, T.G., Entz, T., Parham, J.A., Deng, S.P., Raun, W.R., Johnson, G. V., 2016. Long term effects of lime and phosphorus application on maize productivity in an acid soil of Uasin Gishu. *Journal of Environmental Quality* 5, 48–55. doi:10.2134/jeq2001.301229x
- Orange.biolog, 2014. Orange Data Mining.
- Pearce, Bazin, Lynch, 1995. Environmental Systems, in: *Microbial Biofilms*. p. 208.
- Peigné, J., Vian, J.-F., Cannavacciuolo, M., Bottollier, B., Chaussod, R., 2009. Soil sampling based on field spatial variability of soil microbial indicators. *European Journal of Soil Biology* 45, 488–495. doi:10.1016/j.ejsobi.2009.09.002
- PerkinElmer, 2011. 2400 Series II CHNS/O Elemental Analysis. Perkin Elmer.
- Pester, M., Knorr, K.H., Friedrich, M.W., Wagner, M., Loy, A., 2012. Sulfate-reducing microorganisms in wetlands - fameless actors in carbon cycling and climate change. *Frontiers in Microbiology* 3, 1–19. doi:10.3389/fmicb.2012.00072

- Peter, P., Martha, J., Letcher, M., 2014. Phylogeny and Characterization of Freshwaer Chytridiomycota (Chytridiomycetes and Monoblepharidomycetes), in: Garett Hyde.D Kevin, Pang ka-Lai, J.E.B. (Ed.), *Freshwater Fungi and Fungi-Like Organisms*. pp. 133–149.
- Pfister.H, D., 2015. Pezizomycotina: Pezizomycetes, Orbiliomycetes, in: *Systematics and Evolution Part B*. Springer, pp. 35–55.
- Piao, Z., Yang, L., Zhao, L., Yin, S., 2008. Actinobacterial community structure in soils receiving long-term organic and inorganic amendments. *Applied and Environmental Microbiology* 74, 526–530. doi:10.1128/AEM.00843-07
- Pimentel, D., Terhune, E.C., Dyson-hudson, R., Rochereau, S., Samis, R., Smith, E.A., Denman, D., Reifschneider, D., Shepard, M., Pimentel, D., Terhune, E.C., Dyson-hudson, R., Rochereau, S., Samis, R., Smith, E.A., Denman, D., Reifschneider, D., Shepard, M., 1976. *Land Degradation : Effects on Food and Energy Resources*. Science 194, 149–155.
- Plante, A.F., Pernes, M., Chenu, C., 2005. Changes in clay-associated organic matter quality in a C depletion sequence as measured by differential thermal analyses. *Geoderma* 129, 186–199. doi:10.1016/j.geoderma.2004.12.043
- Plaza-Bonilla, D., Cantero-Martínez, C., Álvaro-Fuentes, J., 2010. Tillage effects on soil aggregation and soil organic carbon profile distribution under Mediterranean semi-arid conditions. *Soil Use and Management* 26, 465–474. doi:10.1111/j.1475-2743.2010.00298.x
- Porter, T.M., Brian Golding, G., 2011. Are similarity- or phylogeny-based methods more appropriate for classifying internal transcribed spacer (ITS) metagenomic amplicons? *New Phytologist* 192, 775–782. doi:10.1111/j.1469-8137.2011.03838.x
- Poulin, M., Darveau, M., 2016. Consequences of delaying conservation of ecosystem services in remote landscapes prone to natural resource exploitation 825–842. doi:10.1007/s10980-015-0291-4
- Powlson, D., 2001. The role of soil microorganisms in soil organic matter conservation in the tropics. *Nutrient Cycling in Agroecosystems* 61, 41–51. doi:10.1023/a:1013338028454
- Pradesh, U., 2010. Molecular Mechanisms of Plant and Microbe Coexistence, in: Nautiyal, C.S., Dioan, P. (Eds.), *Soil Biology*.
- Prosser, J.I., 2012. Nitrification, in: Huang Pan, M., Li, Y., Sumner.E, M. (Eds.), *Handbook of Soil Sciences: Properties and Processes*. CRC Press, pp. 23–27.
- Quastel, J.H., 1965. *Soil metabolism*.
- Quideau, S.A., McIntosh, A.C.S., Norris, C.E., Lloret, E., Swallow, M.J.B., Hannam, K., 2016. Extraction and Analysis of Microbial Phospholipid Fatty Acids in Soils. *Journal of Visualized Experiments* 1–9. doi:10.3791/54360
- Ramirez, K.S., Craine, J.M., Fierer, N., 2012. Consistent effects of nitrogen amendments on soil microbial communities and processes across biomes. *Global Change Biology* 18, 1918–1927. doi:10.1111/j.1365-2486.2012.02639.x
- Rath, K.M., Rousk, J., 2015. Salt effects on the soil microbial decomposer community and their role in organic carbon cycling: A review. *Soil Biology and Biochemistry* 81, 108–123. doi:10.1016/j.soilbio.2014.11.001
- Ratnayake, R.R., Seneviratne, G., Kulasooriya, S.A., 2013. Effect of soil carbohydrates on nutrient availability in natural forests and cultivated lands in Sri Lanka. *Eurasian Soil Science* 46, 579–586. doi:10.1134/S1064229313050177
- RDP [WWW Document], 1992. . Michigan State University. URL <http://rdp.cme.msu.edu>
- Renker, C., Alpehi, J., Buscot, F., 2003. Soil nematodes associated with the mammal pathogenic

- fungus genus *Malassezia* (Basidiomycota: Ustilaginomycetes) in Central European forests. *Biology and Fertility of Soils* 37, 70–72. doi:10.1007/s00374-002-0556-3
- Rennenberg, H., Dannenmann, M., Gessler, A., Kreuzwieser, J., Simon, J., Papen, H., 2009. Nitrogen balance in forest soils: Nutritional limitation of plants under climate change stresses. *Plant Biology* 11, 4–23. doi:10.1111/j.1438-8677.2009.00241.x
- Resources, N., Service, C., 1999. *Soil Taxonomy*. doi:10.1017/S0016756800045489
- Reth, S., Reichstein, M., Falge, E., 2005. The effect of soil water content, soil temperature, soil pH-value and the root mass on soil CO<sub>2</sub> efflux - A modified model. *Plant and Soil* 268, 21–33. doi:10.1007/s11104-005-0175-5
- Ricketts, R., 2004. An Overview of the Newfoundland and Labrador Agrofood Industry.
- Rousk, J., Bååth, E., Brookes, P.C., Lauber, C.L., Lozupone, C., Caporaso, J.G., Knight, R., Fierer, N., 2010. Soil bacterial and fungal communities across a pH gradient in an arable soil. *The ISME Journal* 4, 1340–1351. doi:10.1038/ismej.2010.58
- Rousk, J., Brookes, P.C., Bååth, E., 2009. Contrasting Soil pH Effects on Fungal and Bacterial Growth Suggest Functional Redundancy in Carbon Mineralization □ † 75, 1589–1596. doi:10.1128/AEM.02775-08
- Rovira, P., Ramón Vallejo, V., 2002. Mineralization of carbon and nitrogen from plant debris, as affected by debris size and depth of burial. *Soil Biology and Biochemistry* 34, 327–339. doi:10.1016/S0038-0717(01)00186-9
- Rumpel, C., Kögel-Knabner, I., 2011. Deep soil organic matter-a key but poorly understood component of terrestrial C cycle. *Plant and Soil* 338, 143–158. doi:10.1007/s11104-010-0391-5
- Russell, M.B., 1957. Physical Properties, in: *Soil*. pp. 31–37.
- Sanborn, P., Lamontagne, L., Hendershot, W., 2011. Podzolic soils of Canada: Genesis, distribution, and classification. *Canadian Journal of Soil Science* 91, 843–880. doi:10.4141/cjss10024
- Sapkota, T.B., 2012. Conservation Tillage Impact on Soil Aggregation , Organic Matter Turnover 141–160. doi:10.1007/978-94-007-4113-3
- Schimel, J.P., Bennett, J.B., 2004. Nitrogen Mineralization: Challenges of a Changing Paradigm. *Ecology* 85, 591–602. doi:10.1890/03-8024
- Schimel, J.P., Schaeffer, S.M., 2012. Microbial control over carbon cycling in soil. *Frontiers in Microbiology* 3, 1–11. doi:10.3389/fmicb.2012.00348
- Schloss, P.D., Handelsman, J., 2006. Toward a census of bacteria in soil. *PLoS Computational Biology* 2, 0786–0793. doi:10.1371/journal.pcbi.0020092
- Schmid, K.M., Ohlrogge, J.B., 1973. Lipid metabolism in plants. *Biochemistry of Lipids, Lipoproteins and Membranes* 20, 287–310.
- Schulten, H.R., Schnitzer, M., 1998. R E V I E W A R T I C L E The chemistry of soil organic nitrogen : a review. *Agriculture* 26, 1–15. doi:http://dx.doi.org/10.1007/s003740050335
- Schutter, M.E., Sandeno, J.M., Dick, R.P., 2001. Seasonal, soil type, and alternative management influences on microbial communities of vegetable cropping systems. *Biology and Fertility of Soils* 34, 397–410. doi:10.1007/s00374-001-0423-7
- Sciences, P., 1981. P R O T E I N A N D F R E E A M I N O A C I D S I N W H E A T G R A I N A S A F F E C T E D B Y S O I L T Y P E S A N D S A L I N I T Y L E V E L S 316, 299–316.
- Segers, R., 1998. Methane production and methane consumption: A review of processes underlying wetland methane fluxes. *Biogeochemistry* 41, 23–51. doi:10.1023/A:1005929032764



- Seo, J.S., Keum, Y.S., Li, Q.X., 2009. Bacterial degradation of aromatic compounds, *International Journal of Environmental Research and Public Health*. doi:10.3390/ijerph6010278
- Sergio, M., Cosart, T.F., Johnson, J. V, Holben, W.E., 2011. Extensive Pylogenetic Analysis, in: de Bruijn, J. F. (Ed.), *Handbook of Molecular Microbial Ecology II: Metagenomics in Different Habitats*. John Wiley & Sons, Inc, pp. 151–164.
- Sharpley, A., 1995. Fate and Transport of Nutrients: Phosphorus [WWW Document]. USDA Natural Resources Conversion Service Wisconsin.
- Shi, X.H., Yang, X.M., Drury, C.F., Reynolds, W.D., Mclaughlin, N.B., Zhang, X.P., 2012. Soil & Tillage Research Impact of ridge tillage on soil organic carbon and selected physical properties of a clay loam in southwestern Ontario. *Soil & Tillage Research* 120, 1–7. doi:10.1016/j.still.2012.01.003
- Shin, K., 1995. Minitab Guide.
- Shirouzu, T., Uno, K., Hosaka, K., Hosoya, T., 2016. Early-diverging wood-decaying fungi detected using three complementary sampling methods. *Molecular Phylogenetics and Evolution* 98, 11–20. doi:10.1016/j.ympev.2016.01.015
- Shivlata, L., Satyanarayana, T., 2015. Thermophilic and alkaliphilic Actinobacteria: Biology and potential applications. *Frontiers in Microbiology* 6, 1–29. doi:10.3389/fmicb.2015.01014
- Shrimant Shridhar, B., 2012. Review: Nitrogen Fixing Microorganisms. *International Journal of Microbiological Research* 3, 46–52. doi:10.5829/idosi.ijmr.2012.3.1.61103
- Siala, a., Hill, I.R., Gray, T.R.G., 1974. Populations of Spore-forming Bacteria in an Acid Forest Soil, with Special Reference to *Bacillus subtilis*. *Journal of General Microbiology* 81, 183–190. doi:10.1099/00221287-81-1-183
- Sinsabaugh, R.L., Hill, B.H., Follstad Shah, J.J., 2009. Ecoenzymatic stoichiometry of microbial organic nutrient acquisition in soil and sediment. *Nature* 462, 795–798. doi:10.1038/nature08632
- Sinsabaugh, R.L., Lauber, C.L., Weintraub, M.N., Ahmed, B., Allison, S.D., Crenshaw, C., Contosta, A.R., Cusack, D., Frey, S., Gallo, M.E., Gartner, T.B., Hobbie, S.E., Holland, K., Keeler, B.L., Powers, J.S., Stursova, M., Takacs-Vesbach, C., Waldrop, M.P., Wallenstein, M.D., Zak, D.R., Zeglin, L.H., 2008. Stoichiometry of soil enzyme activity at global scale. *Ecology Letters* 11, 1252–1264. doi:10.1111/j.1461-0248.2008.01245.x
- Smith, S.E., Read, D.J., 2008. *Mycorrhizal Symbiosis*, Third. ed. Elsevier Ltd.
- Spedding, T.A., Hamel, C., Mehuys, G.R., Madramootoo, C.A., 2004. Soil microbial dynamics in maize-growing soil under different tillage and residue management systems. *Soil Biology and Biochemistry* 36, 499–512. doi:10.1016/j.soilbio.2003.10.026
- Stackerbrant, E., Goebel, B.M., 1994. Taxonomic Note: A Place for DNA-DNA Reassociation and 16S rRNA Sequence Analysis in the Present Species Definition in Bacteriology. *International Journal of Systematic and Evolutionary Microbiology* 44, 846–849. doi:10.1099/00207713-44-4-846
- Stein, L.Y., Klotz, M.G., 2016. The nitrogen cycle. *Current Biology* 26, R94–R98. doi:10.1016/j.cub.2015.12.021
- Steven, B.B., Hesse, C., Gallegos-graves, L.V., Belnap, J., Kuske, C.R., 2015. Fungal Diversity in Biological Soil Crusts of the Colorado Plateau.
- Stevenson, B.A., Hunter, D.W.F., Rhodes, P.L., 2014. Temporal and seasonal change in microbial community structure of an undisturbed, disturbed, and carbon-amended pasture soil. *Soil Biology and Biochemistry* 75, 175–185. doi:10.1016/j.soilbio.2014.04.010

- Stevenson, F.J., 1982. Nitrogen in Agricultural Soils. American Society of Agronomy.
- Stevenson, F.J., 1966. Lipids in soil. *Journal of the American Oil Chemists' Society* 43, 203–210. doi:10.1007/BF02641088
- Stolzy, L.H., Fieuhler, H., 1978. Measurement and Prediction of Anaerobiosis in Soils, in: Nielson, R.D., MacDONALD, J. (Eds.), *Nitrogen in the Environment. Nitrogen Behavior in Field Soil*. Volume: 2. pp. 363–449.
- Strickland, M.S., Lauber, C., Fierer, N., Bradford, M.A., 2009. Testing the functional significance of microbial community composition. *Ecology* 90, 441–451. doi:10.1890/08-0296.1
- Sun, B., Jia, S., Zhang, S., McLaughlin, N.B., Liang, A., Chen, X., Liu, S., Zhang, X., 2016. No tillage combined with crop rotation improves soil microbial community composition and metabolic activity. *Environmental Science and Pollution Research* 23, 6472–6482. doi:10.1007/s11356-015-5812-9
- Szukics, U., Hackl, E., Zechmeister-Boltenstern, S., Sessitsch, A., 2012. Rapid and dissimilar response of ammonia oxidizing archaea and bacteria to nitrogen and water amendment in two temperate forest soils. *Microbiological Research* 167, 103–109. doi:10.1016/j.micres.2011.04.002
- Takahiko Nagano, Y.N., n.d. Cultured and Uncultured Fungal Diversity in Deep-Sea Environment, in: Raghukumar, D.C. (Ed.), *Biology of Marine Fungi (Progress in Molecular and Subcellular Biology Marine Molecular Biotechnology)*. Springer Heidelberg New York Dordrecht London, Panjim, India, p. 180.
- Talbot, J.M., Bruns, T.D., Smith, D.P., Branco, S., Glassman, S.I., Erlandson, S., Vilgalys, R., Peay, K.G., 2013. Independent roles of ectomycorrhizal and saprotrophic communities in soil organic matter decomposition. *Soil Biology and Biochemistry* 57, 282–291. doi:10.1016/j.soilbio.2012.10.004
- Tedersoo, L., Arnold, A.E., Hansen, K., 2013. Novel aspects in the life cycle and biotrophic interactions in Pezizomycetes (Ascomycota, Fungi). *Molecular Ecology* 22, 1488–1493. doi:10.1111/mec.12224
- Tegeder, M., Rentsch, D., 2010. Uptake and partitioning of amino acids and peptides. *Molecular Plant* 3, 997–1011. doi:10.1093/mp/ssq047
- Thomasson, A.J., 1992. World map of the status of human-induced soil degradation, *Geoderma*. doi:10.1016/0016-7061(92)90047-B
- Tiedje, J.M., Sexstone, A.J., Parkin, T.B., Revsbech, N.P., 1984. Anaerobic processes in soil. *Plant and Soil* 76, 197–212. doi:10.1007/BF02205580
- Tiedje, J.M., Sexstone, A.J., Parkin, T.B., Revsbech, N.P., Shelton, D.R., 1984. Anaerobic Processes in Soil. *Developments in Plant and Soil Sciences*, Volume: 11., in: Tinsley, J., Darbyshire, J. (Eds.), *Biological Processes and Soil Fertility*. Springer, Dordrecht, pp. 197–212. doi:https://doi.org/10.1007/978-94-009-6101-2\_18
- TISDALL, J.M., OADES, J.M., 1982. Organic matter and water-stable aggregates in soils. *Journal of Soil Science* 33, 141–163. doi:10.1111/j.1365-2389.1982.tb01755.x
- Tong, J., Miaowen, C., Juhui, J., Jinxian, L., Baofeng, C., 2017. Endophytic fungi and soil microbial community characteristics over different years of phytoremediation in a copper tailings dam of Shanxi, China. *Science of the Total Environment* 574, 881–888. doi:10.1016/j.scitotenv.2016.09.161
- Tong, S.T.Y., Chen, W., 2002. Modeling the relationship between land use and surface water quality 377–393. doi:10.1006/jema.2002.0593

- Tonouchi, A., 2009. Isolation and characterization of a novel facultative anaerobic filamentous fungus from Japanese rice field soil. *International Journal of Microbiology* 2009. doi:10.1155/2009/571383
- Topp, E., Pattey, E., 1997. Soils as sources and sinks for atmospheric methane. *Canadian Journal of Soil Science* 77, 167–177. doi:10.4141/S96-107
- Topp, G., Reynolds, W., Cook, F., Kirby, J., Cater, M., 1997. Physical Attributes of Soil Quality, in: *Soil Quality for Crop Production and Ecosystem Health*. Elsevier, pp. 21–52.
- Torsvik, V.L., Goksoyr, J., 1978. Determination of bacterial DNA in soil. *Soil Biology and Biochemistry* 10, 7–12. doi:10.1016/0038-0717(78)90003-2
- Trivedi, P., Delgado-Baquerizo, M., Anderson, I.C., Singh, B.K., 2016. Response of Soil Properties and Microbial Communities to Agriculture: Implications for Primary Productivity and Soil Health Indicators. *Frontiers in Plant Science* 7, 990. doi:10.3389/fpls.2016.00990
- Ulehlova, B., 1988. The Nitrogen Cycle, in: *Soil Microbial Association: Control of Structures and Functions*. pp. 406–462.
- Unite community [WWW Document], 2012. URL <https://unite.ut.ee>
- Usda, 1994. Soil Phosphorus: Soil Quality Kit- Guides for Educators. United States Department of Agriculture: Natural Resources Conservation Service 1–6.
- USDA, 2016. Soil Organic Matter. Soil Quality Kit: Guides for Educators 1–7.
- USDA, 2011. USDA Agroforestry Strategic Framework 1–2.
- USDA, 2001. Rangeland Soil Quality — Infiltration. Agriculture.
- USDA, N., 2004. Soil Biology and Land Management. Soil Biology and Land Management Table 20.
- USDA Natural Resources Conservation Service, 2011. Soil Quality Indicators: soil pH 7–8.
- USDA Natural Resources Conservation Service Soils [WWW Document], n.d.
- USEPA, 1996. Method 3050B - Acid digestion of sediments, sludges, and soils. 1996 2, 12. doi:10.1117/12.528651
- Utuk, I., 2015. Land Degradation : A Threat to Food Security : A Global Assessment 5, 13–22.
- Van Der Heijden, M.G.A., Bardgett, R.D., Van Straalen, N.M., 2008. The unseen majority: Soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology Letters* 11, 296–310. doi:10.1111/j.1461-0248.2007.01139.x
- Van Eerd, L.L., Congreves, K.A., Hayes, A., Verhallen, A., Hooker, D.C., 2014. Long-term tillage and crop rotation effects on soil quality, organic carbon, and total nitrogen. *Canadian Journal of Soil Science* 94, 303–315. doi:10.4141/cjss2013-093
- Veysey, S., 2015. PE 2400 II CHN / S Operating Instructions.
- Vinolas, L.C., Healey, L.R., Jones, D.L., 2001. Kinetics of soil microbial uptake of free amino acids. *Biology and Fertility of Soils* 33, 67–74. doi:10.1007/s003740000291
- Vitousek, P.M., Aber, J.D., Howarth, R.H., Likens, G.E., Matson, P.A., Schindler, D.W., Schlesinger, W.H., Tilman, D.G., 1997. Human alteration of the global nitrogen cycle: Source and consequences. *Ecol Appl* 7, 737–750. doi:10.1038/n1891
- Vladimir, Z., Daniela, K., Wolfgang, S., 2015. The Role of Cellulose Hydrolyzing Bacteria in the Production of Biogas from Plant Biomass, in: Kamm, B. (Ed.), *Microorganisms on Biorefineries*. Springer Heidelberg NewYork Dordrecht Londen, pp. 336–355.
- Vranova, V., Zahradnickova, H., Janous, D., Skene, K.R., Matharu, A.S., Rejsek, K., Formanek, P., 2012. The significance of D-amino acids in soil, fate and utilization by microbes and plants: Review and identification of knowledge gaps. *Plant and Soil* 354, 21–39.

- doi:10.1007/s11104-011-1059-5
- Waldrop, M.P., Balser, T.C., Firestone, M.K., 2000. Linking microbial community composition to function in a tropical soil. *Soil Biology & Biochemistry* 32, 1837–1846. doi:10.1016/S0038-0717(00)00157-7
- Waldrop, M.P., Firestone, M.K., 2006. Response of microbial community composition and function to soil climate change. *Microbial Ecology* 52, 716–724. doi:10.1007/s00248-006-9103-3
- Wallander, H., Nilsson, L.O., Hagerberg, D., Rosengren, U., 2003. Direct estimates of C:N ratios of ectomycorrhizal mycelia collected from Norway spruce forest soils. *Soil Biology and Biochemistry* 35, 997–999. doi:10.1016/S0038-0717(03)00121-4
- Wallenstein, M.D., McMahon, S.K., Schimel, J.P., 2009. Seasonal variation in enzyme activities and temperature sensitivities in Arctic tundra soils. *Global Change Biology* 15, 1631–1639. doi:10.1111/j.1365-2486.2008.01819.x
- Wardle, D.A., Ghani, A., 1995. A critique of the microbial metabolic quotient (qCO<sub>2</sub>) as a bioindicator of disturbance and ecosystem development. *Soil Biology and Biochemistry* 27, 1601–1610. doi:10.1016/0038-0717(95)00093-T
- Wardle, D.A., Jonsson, M., Bansal, S., Bardgett, R.D., Gundale, M.J., Metcalfe, D.B., 2012. Linking vegetation change, carbon sequestration and biodiversity: Insights from island ecosystems in a long-term natural experiment. *Journal of Ecology* 100, 16–30. doi:10.1111/j.1365-2745.2011.01907.x
- White, T.J., Bruns, S., Lee, S., Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR Protocols: A Guide to Methods and Applications*. doi:citeulike-article-id:671166
- Whitman, W.B., Coleman, D.C., Wiebe, W.J., 1998. Prokaryotes: The unseen majority. *Proceedings of the National Academy of Sciences* 95, 6578–6583. doi:10.1073/pnas.95.12.6578
- Wiederholt, R., Johnson, B., 2005. Nitrogen Behavior.
- Wiesenberg, G.L.B., Gocke, M., Kuzyakov, Y., 2010. Fast incorporation of root-derived lipids and fatty acids into soil - Evidence from a short term multiple <sup>14</sup>CO<sub>2</sub> pulse labelling experiment. *Organic Geochemistry* 41, 1049–1055. doi:10.1016/j.orggeochem.2009.12.007
- Wilkinson, A., Hill, P.W., Farrar, J.F., Jones, D.L., Bardgett, R.D., 2014. Rapid microbial uptake and mineralization of amino acids and peptides along a grassland productivity gradient. *Soil Biology and Biochemistry* 72, 75–83. doi:10.1016/j.soilbio.2014.01.026
- Willems, H.P.L., Lewis, K.J., Dyson, J.S., Lewis, F.J., 1996. Mineralization of 2,4-D and atrazine in the unsaturated zone of a sandy loam soil. *Soil Biology and Biochemistry* 28, 989–996. doi:10.1016/0038-0717(96)00123-X
- Winter, G., Todd, C.D., Trovato, M., Forlani, G., Funck, D., 2015. Physiological implications of arginine metabolism in plants. *Frontiers in Plant Science* 6, 1–14. doi:10.3389/fpls.2015.00534
- Wittmann, C., Kähkönen, M.A., Ilvesniemi, H., Kurola, J., Salkinoja-Salonen, M.S., 2004. Areal activities and stratification of hydrolytic enzymes involved in the biochemical cycles of carbon, nitrogen, sulphur and phosphorus in podsolized boreal forest soils. *Soil Biology and Biochemistry* 36, 425–433. doi:10.1016/j.soilbio.2003.10.019
- Wolińska, A., Szafranek-Nakonieczna, A., Banach, A., Błaszczuk, M., Stępniewska, Z., 2016. The impact of agricultural soil usage on activity and abundance of ammonifying bacteria in selected soils from Poland. *SpringerPlus* 5, 565. doi:10.1186/s40064-016-2264-8

- Wu, L., Wen, C., Qin, Y., Yin, H., Tu, Q., Van Nostrand, J.D., Yuan, T., Yuan, M., Deng, Y., Zhou, J., 2015. Phasing amplicon sequencing on Illumina Miseq for robust environmental microbial community analysis. *BMC Microbiology* 15, 125. doi:10.1186/s12866-015-0450-4
- Wu, Y., Ke, X., Hernández, M., Wang, B., Dumont, M.G., Jia, Z., Conrad, R., 2013. Autotrophic growth of bacterial and archaeal ammonia oxidizers in freshwater sediment microcosms incubated at different temperatures. *Applied and Environmental Microbiology* 79, 3076–3084. doi:10.1128/AEM.00061-13
- Wu, Z., Tsumura, Y., Blomquist, G., Wang, X., 2003. 18S rRNA gene variation among common airborne fungi, and development of specific oligonucleotide probes for the detection of fungal isolates. *Applied and Environmental Microbiology* 69, 5389–97. doi:10.1128/AEM.69.9.5389
- Wu, Z., Wang, X.-R., Blomquist, G., 2002. Evaluation of PCR primers and PCR conditions for specific detection of common airborne fungi. *Journal of Environmental Monitoring: JEM* 4, 377–382. doi:10.1039/b200490a
- Xia, F.F., Su, Y., Wei, X.M., He, Y.H., Wu, Z.C., Ghulam, A., He, R., 2014. Diversity and activity of sulphur-oxidizing bacteria and sulphate-reducing bacteria in landfill cover soils. *Letters in Applied Microbiology* 59, 26–34. doi:10.1111/lam.12240
- Yabe, S., Sakai, Y., Abe, K., Yokota, A., 2017. Diversity of Ktedonobacteria with Actinomycetes-Like Morphology in Terrestrial Environments. *Microbes Environ* 32, 61–70. doi:10.1264/jsme2.ME16144
- Yu, A.Z., Zhang, Q., Kraus, T.E.C., Dahlgren, R.A., Anastasio, C., Zasoski, R.J., 2010. Contribution of amino compounds to dissolved organic nitrogen in forest soils 61, 173–198.
- Yu, C., Hu, X., Deng, W., Li, Y., Han, G., Xiong, C., 2016. Response of bacteria community to long-term inorganic nitrogen application in mulberry field soil. *PLoS ONE* 11, 1–12. doi:10.1371/journal.pone.0168152
- Yu, H., Ding, W., Luo, J., Geng, R., Cai, Z., 2012. Long-term application of organic manure and mineral fertilizers on aggregation and aggregate-associated carbon in a sandy loam soil. *Soil and Tillage Research* 124, 170–177. doi:10.1016/j.still.2012.06.011
- Yurkov, A.M., Röhl, O., Pontes, A., Carvalho, C., Maldonado, C., Sampaio, J.P., 2015. Local climatic conditions constrain soil yeast diversity patterns in mediterranean forests, woodlands and scrub biome. *FEMS Yeast Research* 16, 1–11. doi:10.1093/femsyr/fov103
- Zalar, P., Sybren de Hoog, G., Schroers, H.J., Frank, J.M., Gunde-Cimerman, N., 2005. Taxonomy and phylogeny of the xerophilic genus *Wallemia* (Wallemiomycetes and Wallemiales, cl. et ord. nov.). *Antonie van Leeuwenhoek, International Journal of General and Molecular Microbiology* 87, 311–328. doi:10.1007/s10482-004-6783-x
- Zehr, J.P., Jenkins, B.D., Short, S.M., Steward, G.F., 2003. Nitrogenase gene diversity and microbial community structure: a cross-system comparison. *Environ Microbiol* 5, 539–554. doi:10.1046/j.1462-2920.2003.00451.x
- Zenova, G.M., Gryadunova, A.A., Doroshenko, E.A., Likhacheva, A.A., Sudnitsyn, I.I., Pochatkova, T.N., Zvyagintsev, D.G., 2007. Influence of moisture on the vital activity of actinomycetes in a cultivated low-moor peat soil. *Eurasian Soil Science* 40, 560–564. doi:10.1134/S1064229307050110
- Zhang, Q., Dick, W.A., 2014. Growth of soil bacteria, on penicillin and neomycin, not previously exposed to these antibiotics. *Science of the Total Environment* 493, 445–453. doi:10.1016/j.scitotenv.2014.05.114

- Zornoza, R., Guerrero, C., Mataix-Solera, J., Scow, K.M., Arcenegui, V., Mataix-Beneyto, J., 2009. Changes in soil microbial community structure following the abandonment of agricultural terraces in mountainous areas of Eastern Spain. *Applied Soil Ecology : A Section of Agriculture, Ecosystems & Environment* 42, 315–323.  
doi:10.1016/j.apsoil.2009.05.011
- Zou, Y., Zhang, J., Yang, D., Chen, X., Zhao, J., Xiu, W., Lai, X., Li, G., 2011. Effects of different land use patterns on nifH genetic diversity of soil nitrogen-fixing microbial communities in *Leymus Chinensis* steppe. *Acta Ecologica Sinica* 31, 150–156.  
doi:10.1016/j.chnaes.2011.03.004
- Zverlow, V., Daniela, K., Wolfgang, S., 2015. The Role of Cellulose-Hydrolyzing Bacteria in the Production of Biogas from Plant Biomass, in: Kamm, B. (Ed.), *Microorganisms on Biorefineries*. Springer -Verlag Berlin Heidelberg, p. 345.